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<b>(54) Title:</b> NOVEL HUMAN $\beta_2$ INTEGRIN ALPHA SUBUNIT  <b>(57) Abstract</b>  DNA encoding a novel human $\beta_2$ integrin $\alpha$ subunit polypeptide, designated $\alpha_4$ , is disclosed along with methods and materials for production of the same by recombinant procedures. Fusion proteins are also disclosed which include extracellular $\alpha_4$ protein fragments, $\alpha_4$ I domain fragments or full length $\alpha_4$ polypeptides and human immunoglobulin constant regions. Binding molecules specific for $\alpha_4$ are also disclosed as useful for modulating the biological activities of $\alpha_4$ . DNA from other species which show homology to human $\alpha_4$ encoding sequences are also disclosed.		

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## Novel Human $\beta_2$ Integrin Alpha Subunit

This application is a continuation-in-part of U.S. Application Serial No. 08/286,889, filed August 5, 1994, which is pending, which in turn is a continuation-in-part of U.S. Application Serial No. 08/173,497, filed December 23, 1993, which is pending.

### Field of the Invention

The present invention relates to the cloning and expression of polynucleotides encoding a novel human  $\beta_2$  integrin  $\alpha$  subunit, designated  $\alpha_d$ , which is structurally related to the known human  $\beta_2$  integrin  $\alpha$  subunits, CD11a, CD11b and CD11c. The present invention also relates to polynucleotides isolated from other species which show homology to human  $\alpha_d$  encoding sequences.

### Background of the Invention

The integrins are a class of membrane-associated molecules which actively participate in cellular adhesion. Integrins are transmembrane heterodimers comprising an  $\alpha$  subunit in noncovalent association with a  $\beta$  subunit. To date, at least fourteen  $\alpha$  subunits and eight  $\beta$  subunits have been identified [reviewed in Springer, *Nature* 346:425-434 (1990)]. The  $\beta$  subunits are generally capable of association with more than one  $\alpha$  subunit and the heterodimers sharing a common  $\beta$  subunit have been classified as subfamilies within the integrin population.

One class of human integrins, restricted to expression in white blood cells, is characterized by a common  $\beta_2$  subunit. As a result of this cell-specific expression, these integrins are commonly referred to as the leukocyte integrins, Leu-CAMs or leukointegrins. Because of the common  $\beta_2$  subunit, an alternative designation of this class is the  $\beta_2$  integrins. The  $\beta_2$  subunit (CD18) has previously been isolated in association with one of three distinct  $\alpha$  subunits,

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CD11a, CD11b or CD11c. The isolation of a cDNA encoding human CD18 is described in Kishimoto, *et al.*, *Cell* 48:681-690 (1987). In official WHO nomenclature, the heterodimeric proteins are referred to as CD11a/CD18, CD11b/CD18, and CD11c/CD18; in common nomenclature they are referred to as LFA-1, Mac-1 or Mo1 and p150,95 or LeuM5, respectively [Cobbold, *et al.*, in *Leukocyte Typing III*, McMichael (ed), Oxford Press, p.788 (1987)]. The human  $\beta_2$  integrin  $\alpha$  subunits CD11a, CD11b and CD11c have been demonstrated to migrate under reducing condition in electrophoresis with apparent molecular weights of approximately 180 kD, 155 kD and 150 kD, respectively, and DNAs encoding these subunits have been cloned [CD11a, Larson, *et al.*, *J. Cell Biol.* 108:703-712 (1989); CD11b, Corbi, *et al.*, *J. Biol. Chem.* 263:12403-12411 (1988) and CD11c, Corbi, *et al.*, *EMBO J.* 6:4023-4028 (1987)]. Putative homologs of the human  $\beta_2$  integrin  $\alpha$  and  $\beta$  chains, defined by approximate similarity in molecular weight, have been variously identified in other species including monkeys and other primates [Letvin, *et al.*, *Blood* 61:408-410 (1983)], mice [Sanchez-Madrid, *et al.*, *J. Exp. Med.* 154:1517 (1981)], and dogs [Moore, *et al.*, *Tissue Antigens* 36:211-220 (1990)].

The absolute molecular weights of presumed homologs from other species have been shown to vary significantly [see, *e.g.*, Danilenko *et al.*, *Tissue Antigens* 40:13-21 (1992)], and in the absence of sequence information, a definitive correlation between human integrin subunits and those identified in other species has not been possible. Moreover, variation in the number of members in a protein family has been observed between different species. Consider, for example, that more IgA isotypes have been isolated in rabbits than in humans [Burnett, *et al.*, *EMBO J.* 8:4041-4047 (1989) and Schneiderman, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 86:7561-7565 (1989)]. Similarly, in humans, at least six variants of the metallothioneine protein have been previously identified [Karin and Richards, *Nature* 299:797-802 (1982) and Varshney, *et al.*, *Mol. Cell. Biol.* 6:26-37, (1986)], whereas in the mouse, only two such variants are

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in evidence [Searle, *et al.*, *Mol.Cell.Biol.* 4:1221-1230 (1984)]. Therefore, existence of multiple members of a protein family in one species does not necessarily imply that corresponding family members exist in another species.

5 In the specific context of  $\beta_2$  integrins, in dogs it has been observed that the presumed canine  $\beta_2$  counterpart to the human CD18 is capable of dimer formation with as many as four potentially distinct  $\alpha$  subunits [Danilenko, *et al.*, *supra*]. Antibodies generated by immunizing mice with canine splenocytes resulted in monoclonal antibodies which immunoprecipitated proteins tentatively designated as canine homologs to human CD18, CD11a, CD11b and CD11c based  
10 mainly on similar, but not identical, molecular weights. Another anti-canine splenocyte antibody, Ca11.8H2, recognized and immunoprecipitated a fourth  $\alpha$ -like canine subunit also capable of association with the  $\beta_2$  subunit, but having a unique molecular weight and restricted in expression to a subset of differentiated tissue macrophages. Antibodies generated by immunization of hamsters with  
15 murine dendritic cells resulted in two anti-integrin antibodies [Metlay, *et al.*, *J.Exp.Med.* 171:1753-1771 (1990)]. One antibody, 2E6, immunoprecipitated a predominant heterodimer with subunits having approximate molecular weights of 180 kD and 90 kD in addition to minor bands in the molecular weight range of 150-160 kD. The second antibody, N418, precipitated another apparent  
20 heterodimer with subunits having approximate molecular weights of 150 kD and 90 Kd. Based on cellular adhesion blocking studies, it was hypothesized that antibody 2E6 recognized a murine counterpart to human CD18. While the molecular weight of the N418 antigen suggested recognition of a murine homolog to human CD11c/CD18, further analysis indicated that the murine antigen  
25 exhibited a tissue distribution pattern which was inconsistent with that observed for human CD11c/CD18.

The antigens recognized by the canine Ca11.8H2 antibody and the murine N418 antibody could represent a variant species (*e.g.*, a glycosylation or splice variant) of a previously identified canine or murine  $\alpha$  subunit.

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Alternatively, these antigens may represent unique canine and murine integrin  $\alpha$  subunits. In the absence of specific information regarding primary structure, these alternatives cannot be distinguished.

In humans, CD11a/CD18 is expressed on all leukocytes. CD11b/CD18 and CD11c/CD18 are essentially restricted to expression on monocytes, granulocytes, macrophages and natural killer (NK) cells, but CD11c/CD18 is also detected on some B-cell types. In general, CD11a/CD18 predominates on lymphocytes, CD11b/CD18 on granulocytes and CD11c/CD18 on macrophages [see review, Arnaut, *Blood* 75:1037-1050 (1990)]. Expression of the  $\alpha$  chains, however, is variable with regard to the state of activation and differentiation of the individual cell types [See review, Larson and Springer, *Immunol.Rev.* 114:181-217 (1990).]

The involvement of the  $\beta_2$  integrins in human immune and inflammatory responses has been demonstrated using monoclonal antibodies which are capable of blocking  $\beta_2$  integrin-associated cell adhesion. For example, CD11a/CD18, CD11b/CD18 and CD11c/CD18 actively participate in natural killer (NK) cell binding to lymphoma and adenocarcinoma cells [Patarroyo, *et al.*, *Immunol.Rev.* 114:67-108 (1990)], granulocyte accumulation [Nourshargh, *et al.*, *J.Immunol.* 142:3193-3198 (1989)], granulocyte-independent plasma leakage [Arfors, *et al.*, *Blood* 69:338-340 (1987)], chemotactic response of stimulated leukocytes [Arfors, *et al.*, *supra*] and leukocyte adhesion to vascular endothelium [Price, *et al.*, *J.Immunol.* 139:4174-4177 (1987) and Smith, *et al.*, *J.Clin.Invest.* 83:2008-2017 (1989)]. The fundamental role of  $\beta_2$  integrins in immune and inflammatory responses is made apparent in the clinical syndrome referred to as leukocyte adhesion deficiency (LAD), wherein clinical manifestations include recurrent and often life threatening bacterial infections. LAD results from heterogeneous mutations in the  $\beta_2$  subunit [Kishimoto, *et al.*, *Cell* 50:193-202 (1987)] and the severity of the disease state is proportional to the degree of the



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deficiency in  $\beta_2$  subunit expression. Formation of the complete integrin heterodimer is impaired by the  $\beta_2$  mutation [Kishimoto, *et al.*, *supra*].

Interestingly, at least one antibody specific for CD18 has been shown to inhibit human immunodeficiency virus type-1 (HIV-1) syncytia formation *in vitro*, albeit the exact mechanism of this inhibition is unclear [Hildreth and Orentas, *Science* 244:1075-1078 (1989)]. This observation is consistent with the discovery that a principal counterreceptor of CD11a/CD18, ICAM-1, is also a surface receptor for the major group of rhinovirus serotypes [Greve, *et al.*, *Cell* 56:839 (1989)].

The significance of  $\beta_2$  integrin binding activity in human immune and inflammatory responses underscores the necessity to develop a more complete understanding of this class of surface proteins. Identification of yet unknown members of this subfamily, as well as their counterreceptors, and the generation of monoclonal antibodies or other soluble factors which can alter biological activity of the  $\beta_2$  integrins will provide practical means for therapeutic intervention in  $\beta_2$  integrin-related immune and inflammatory responses.

#### Brief Description of the Invention

In one aspect, the present invention provides novel purified and isolated polynucleotides (*e.g.*, DNA and RNA transcripts, both sense and anti-sense strands) encoding a novel human  $\beta_2$  integrin  $\alpha$  subunit,  $\alpha_d$ , and variants thereof (*i.e.*, deletion, addition or substitution analogs) which possess binding and/or immunological properties inherent to  $\alpha_d$ . Preferred DNA molecules of the invention include cDNA, genomic DNA and wholly or partially chemically synthesized DNA molecules. A presently preferred polynucleotide is the DNA as set forth in SEQ ID NO: 1, encoding the polypeptide of SEQ ID NO: 2. Also provided are recombinant plasmid and viral DNA constructions (expression constructs) which include  $\alpha_d$  encoding sequences, wherein the  $\alpha_d$  encoding

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sequence is operatively linked to a homologous or heterologous transcriptional regulatory element or elements.

Also provided by the present invention are isolated and purified mouse and rat polynucleotides which exhibit homology to polynucleotides encoding human  $\alpha_d$ . A preferred mouse polynucleotide is set forth in SEQ ID NO: 52; a preferred rat polynucleotide is set forth in SEQ ID NO: 54.

As another aspect of the invention, prokaryotic or eukaryotic host cells transformed or transfected with DNA sequences of the invention are provided which express  $\alpha_d$  polypeptide or variants thereof. Host cells of the invention are particularly useful for large scale production of  $\alpha_d$  polypeptide, which can be isolated from either the host cell itself or from the medium in which the host cell is grown. Host cells which express  $\alpha_d$  polypeptide on their extracellular membrane surface are also useful as immunogens in the production of  $\alpha_d$ -specific antibodies. Preferably, host cells transfected with  $\alpha_d$  will be co-transfected to express a  $\beta_2$  integrin subunit in order to allow surface expression of the heterodimer.

Also provided by the present invention are purified and isolated  $\alpha_d$  polypeptides, fragments and variants thereof. Preferred  $\alpha_d$  polypeptides are as set forth in SEQ ID NO: 2. Novel  $\alpha_d$  products of the invention may be obtained as isolates from natural sources, but, along with  $\alpha_d$  variant products, are preferably produced by recombinant procedures involving host cells of the invention. Completely glycosylated, partially glycosylated and wholly de-glycosylated forms of the  $\alpha_d$  polypeptide may be generated by varying the host cell selected for recombinant production and/or post-isolation processing. Variant  $\alpha_d$  polypeptides of the invention may comprise water soluble and insoluble  $\alpha_d$  polypeptides including analogs wherein one or more of the amino acids are deleted or replaced: (1) without loss, and preferably with enhancement, of one or more biological activities or immunological characteristics specific for  $\alpha_d$ ; or (2) with specific disablement of a particular ligand/receptor binding or signalling

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function. Fusion polypeptides are also provided, wherein  $\alpha_d$  amino acid sequences are expressed contiguously with amino acid sequences from other polypeptides. Such fusion polypeptides may possess modified biological, biochemical, and/or immunological properties in comparison to wild-type  $\alpha_d$ .  
5 Analog polypeptides including additional amino acid (e.g., lysine or cysteine) residues that facilitate multimer formation are contemplated.

Also comprehended by the present invention are polypeptides and other non-peptide molecules which specifically bind to  $\alpha_d$ . Preferred binding molecules include antibodies (e.g., monoclonal and polyclonal antibodies),  
10 counterreceptors (e.g., membrane-associated and soluble forms) and other ligands (e.g., naturally occurring or synthetic molecules), including those which competitively bind  $\alpha_d$  in the presence of  $\alpha_d$  monoclonal antibodies and/or specific counterreceptors. Binding molecules are useful for purification of  $\alpha_d$  polypeptides and identifying cell types which express  $\alpha_d$ . Binding molecules are also useful for  
15 modulating (i.e., inhibiting, blocking or stimulating) of *in vivo* binding and/or signal transduction activities of  $\alpha_d$ .

Assays to identify  $\alpha_d$  binding molecules are also provided, including immobilized ligand binding assays, solution binding assays, scintillation proximity assays, di-hybrid screening assays, and the like.

20 *In vitro* assays for identifying antibodies or other compounds that modulate the activity of  $\alpha_d$  may involve, for example, immobilizing  $\alpha_d$  or a natural ligand to which  $\alpha_d$  binds, detectably labelling the nonimmobilized binding partner, incubating the binding partners together and determining the effect of a test compound on the amount of label bound wherein a reduction in the label  
25 bound in the presence of the test compound compared to the amount of label bound in the absence of the test compound indicates that the test agent is an inhibitor of  $\alpha_d$  binding.

Another type of assay for identifying compounds that modulate the interaction between  $\alpha_d$  and a ligand involves immobilizing  $\alpha_d$  or a fragment

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thereof on a solid support coated (or impregnated with) a fluorescent agent, labelling the ligand with a compound capable of exciting the fluorescent agent, contacting the immobilized  $\alpha_d$  with the labelled ligand in the presence and absence of a putative modulator compound, detecting light emission by the fluorescent agent, and identifying modulating compounds as those compounds that affect the emission of light by the fluorescent agent in comparison to the emission of light by the fluorescent agent in the absence of a modulating compound. Alternatively, the  $\alpha_d$  ligand may be immobilized and  $\alpha_d$  may be labelled in the assay.

Yet another method contemplated by the invention for identifying compounds that modulate the interaction between  $\alpha_d$  and a ligand involves transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain, expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of  $\alpha_d$  and either the DNA binding domain or the activating domain of the transcription factor, expressing in the host cells a second hybrid DNA sequence encoding part or all of the ligand and the DNA binding domain or activating domain of the transcription factor which is not incorporated in the first fusion, evaluating the effect of a putative modulating compound on the interaction between  $\alpha_d$  and the ligand by detecting binding of the ligand to  $\alpha_d$  in a particular host cell by measuring the production of reporter gene product in the host cell in the presence or absence of the putative modulator, and identifying modulating compounds as those compounds altering production of the reported gene product in comparison to production of the reporter gene product in the absence of the modulating compound. Presently preferred for use in the assay are the *lexA* promoter, the *lexA* DNA binding domain, the GAL4 transactivation domain, the *lacZ* reporter gene, and a yeast host cell.

A modified version of the foregoing assay may be used in isolating a polynucleotide encoding a protein that binds to  $\alpha_d$  by transforming or

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transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain, expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of  $\alpha_d$  and either the DNA binding domain or the activating domain of the transcription factor, expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative  $\alpha_d$  binding proteins and the DNA binding domain or activating domain of the transcription factor which is not incorporated in the first fusion, detecting binding of an  $\alpha_d$  binding protein to  $\alpha_d$  in a particular host cell by detecting the production of reporter gene product in the host cell, and isolating second hybrid DNA sequences encoding  $\alpha_d$  binding protein from the particular host cell.

Hybridoma cell lines which produce antibodies specific for  $\alpha_d$  are also comprehended by the invention. Techniques for producing hybridomas which secrete monoclonal antibodies are well known in the art. Hybridoma cell lines may be generated after immunizing an animal with purified  $\alpha_d$ , variants of  $\alpha_d$  or cells which express  $\alpha_d$  or a variant thereof on the extracellular membrane surface. Immunogen cell types include cells which express  $\alpha_d$  *in vivo*, or transfected prokaryotic or eukaryotic cell lines which normally do not normally express  $\alpha_d$  *in vivo*.

The value of the information contributed through the disclosure of the DNA and amino acid sequences of  $\alpha_d$  is manifest. In one series of examples, the disclosed  $\alpha_d$  cDNA sequence makes possible the isolation of the human  $\alpha_d$  genomic DNA sequence, including transcriptional control elements for the genomic sequence. Identification of  $\alpha_d$  allelic variants and heterologous species (*e.g.*, rat or mouse) DNAs is also comprehended. Isolation of the human  $\alpha_d$  genomic DNA and heterologous species DNAs can be accomplished by standard DNA/DNA hybridization techniques, under appropriately stringent conditions, using all or part of the  $\alpha_d$  cDNA sequence as a probe to screen an appropriate

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library. Alternatively, polymerase chain reaction (PCR) using oligonucleotide primers that are designed based on the known cDNA sequence can be used to amplify and identify genomic  $\alpha_d$  DNA sequences. Synthetic DNAs encoding the  $\alpha_d$  polypeptide, including fragments and other variants thereof, may be produced by conventional synthesis methods.

DNA sequence information of the invention also makes possible the development, by homologous recombination or "knockout" strategies [see, *e.g.*, Kamekuchi, *Science* 244:1288-1292 (1989)], to produce rodents that fail to express a functional  $\alpha_d$  polypeptide or that express a variant  $\alpha_d$  polypeptide. Such rodents are useful as models for studying the activities of  $\alpha_d$  and  $\alpha_d$  modulators *in vivo*.

DNA and amino acid sequences of the invention also make possible the analysis of  $\alpha_d$  epitopes which actively participate in counterreceptor binding as well as epitopes which may regulate, rather than actively participate in, binding. Identification of epitopes which may participate in transmembrane signal transduction is also comprehended by the invention.

DNA of the invention is also useful for the detection of cell types which express  $\alpha_d$  polypeptide. Standard DNA/RNA hybridization techniques which utilize  $\alpha_d$  DNA to detect  $\alpha_d$  RNA may be used to determine the constitutive level of  $\alpha_d$  transcription within a cell, as well as changes in the level of transcription in response to internal or external agents. Identification of agents which modify transcription and/or translation of  $\alpha_d$  can, in turn, be assessed for potential therapeutic or prophylactic value. DNA of the invention also makes possible *in situ* hybridization of  $\alpha_d$  DNA to cellular RNA to determine the cellular localization of  $\alpha_d$  specific messages within complex cell populations and tissues.

DNA of the invention is also useful for identification of non-human polynucleotide sequences which display homology to human  $\alpha_d$  sequences. Possession of non-human  $\alpha_d$  DNA sequences permits development of animal models (including, for example, transgenic models) of the human system.

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As another aspect of the invention, monoclonal or polyclonal antibodies specific for  $\alpha_d$  may be employed in immunohistochemical analysis to localize  $\alpha_d$  to subcellular compartments or individual cells within tissues. Immunohistochemical analyses of this type are particularly useful when used in combination with *in situ* hybridization to localize both  $\alpha_d$  mRNA and polypeptide products of the  $\alpha_d$  gene.

Identification of cell types which express  $\alpha_d$  may have significant ramifications for development of therapeutic and prophylactic agents. It is anticipated that the products of the invention related to  $\alpha_d$  can be employed in the treatment of diseases wherein macrophages are an essential element of the disease process. Animal models for many pathological conditions associated with macrophage activity have been described in the art. For example, in mice, macrophage recruitment to sites of both chronic and acute inflammation is reported by Jutila, *et al.*, [*J. Leukocyte Biol.* 54:30-39 (1993)]. In rats, Adams, *et al.*, [*Transplantation* 53:1115-1119 (1992) and *Transplantation* 56:794-799 (1993)] describe a model for graft arteriosclerosis following heterotopic abdominal cardiac allograft transplantation. Rosenfeld, *et al.*, [*Arteriosclerosis* 7:9-23 (1987) and *Arteriosclerosis* 7:24-34 (1987)] describe induced atherosclerosis in rabbits fed a cholesterol supplemented diet. Hanenberg, *et al.*, [*Diabetologia* 32:126-134 (1989)] report the spontaneous development of insulin-dependent diabetes in BB rats. Yamada *et al.*, [*Gastroenterology* 104:759-771 (1993)] describe an induced inflammatory bowel disease, chronic granulomatous colitis, in rats following injections of streptococcal peptidoglycan-polysaccharide polymers. Cromartie, *et al.*, [*J. Exp. Med.* 146:1585-1602 (1977)] and Schwab, *et al.*, [*Infection and Immunity* 59:4436-4442 (1991)] report that injection of streptococcal cell wall protein into rats results in an arthritic condition characterized by inflammation of peripheral joints and subsequent joint destruction. Finally, Huitinga, *et al.*, [*Eur. J. Immunol.* 23:709-715 (1993)] describe experimental allergic encephalomyelitis, a model for multiple sclerosis, in Lewis rats. In each of these

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models,  $\alpha_d$  antibodies, other  $\alpha_d$  binding proteins, or soluble forms of  $\alpha_d$  are utilized to attenuate the disease state, presumably through inactivation of macrophage activity.

5        Pharmaceutical compositions for treatment of these and other disease states are provided by the invention. Pharmaceutical compositions are designed for the purpose of inhibiting interaction between  $\alpha_d$  and its ligand(s) and include various soluble and membrane-associated forms of  $\alpha_d$  (comprising the entire  $\alpha_d$  polypeptide, or fragments thereof which actively participate in  $\alpha_d$  binding), soluble and membrane-associated forms of  $\alpha_d$  binding proteins  
10        (including antibodies, ligands, and the like), intracellular or extracellular modulators of  $\alpha_d$  binding activity, and/or modulators of  $\alpha_d$  and/or  $\alpha_d$ -ligand polypeptide expression, including modulators of transcription, translation, post-translational processing and/or intracellular transport. The invention also comprehends methods for treatment of disease states in which  $\alpha_d$  binding is  
15        implicated, wherein a patient suffering from said disease state is provided an amount of a pharmaceutical composition of the invention sufficient to modulate levels of  $\alpha_d$  binding. The method of treatment of the invention is applicable to disease states such as, but not limited to, Type I diabetes, atherosclerosis, multiple sclerosis, asthma, psoriasis, and rheumatoid arthritis.

20        **Brief Description of the Drawing**

Numerous other aspects and advantages of the present invention will be apparent upon consideration of the following description thereof, reference being made to the drawing wherein:

25        Figure 1A through 1D comprises an alignment of the human amino acid sequences of CD11b (SEQ ID NO: 3), CD11c (SEQ ID NO: 4) and  $\alpha_d$  (SEQ ID NO: 2).



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**Detailed Description of the Invention**

The present invention is illustrated by the following examples relating to the isolation of a cDNA clone encoding  $\alpha_d$  from a human spleen cDNA library. More particularly, Example 1 illustrates the use of anti-canine  $\alpha_{TM1}$  antibody in an attempt to detect a homologous human protein. Example 2 details purification of canine  $\alpha_{TM1}$  and N-terminal sequencing of the polypeptide to design oligonucleotide primers for PCR amplification of the canine  $\alpha_{TM1}$  gene. Example 3 addresses large scale purification of canine  $\alpha_{TM1}$  for internal sequencing in order to design additional PCR primers. Example 4 describes use of the PCR and internal sequence primers to amplify a fragment of the canine  $\alpha_{TM1}$  gene. Example 5 addresses cloning of the human  $\alpha_d$ -encoding cDNA sequence. Example 6 describes Northern blot hybridization analysis of human tissues and cells for expression of  $\alpha_d$  mRNA. Example 7 details the construction of human  $\alpha_d$  expression plasmids and transfection of COS cells with the resulting plasmids. Example 8 addresses ELISA analysis of  $\alpha_d$  expression in transfected COS cells. Example 9 describes FACS analysis of COS cells transfected with human  $\alpha_d$  expression plasmids. Example 10 addresses immunoprecipitation of CD18 in association with  $\alpha_d$  in co-transfected COS cells. Example 11 relates to stable transfection of  $\alpha_d$  expression constructs in Chinese hamster ovary cells. Example 12 addresses CD18-dependent binding of  $\alpha_d$  to the intercellular adhesion molecule, ICAM-R. Example 13 describes scintillation proximity screening assays to identify inhibitors of  $\alpha_d$  ligand/anti-ligand binding interactions. Example 14 addresses construction of expression plasmids which encode soluble forms of  $\alpha_d$ . Example 15 relates to production of  $\alpha_d$ -specific monoclonal antibodies. Example 16 describes analysis of  $\alpha_d$  tissue distribution using polyclonal antiserum. Example 17 describes isolation of rat cDNA sequences which show homology to human  $\alpha_d$  gene sequences. Example 18 relates to construction of rat  $\alpha_d$  I domain expression plasmids, including I domain/IgG fusion proteins, and production of monoclonal antibodies to I domain fusion

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proteins. Example 19 addresses isolation of mouse cDNA sequences which show homology to human  $\alpha_d$  gene sequences. Example 20 describes isolation of additional mouse  $\alpha_d$  cDNA clones used for conformational sequence analysis. Example 21 relates to *in situ* hybridization analysis of various mouse tissues to determine tissue and cell specific expression of the putative mouse homolog to human  $\alpha_d$ . Example 22 describes generation of expression constructs which encode the putative mouse homolog of human  $\alpha_d$ . Example 23 addresses design of a "knock-out" mouse wherein the gene encoding the putative mouse homolog of human  $\alpha_d$  is disrupted. Example 24 describes isolation of rabbit cDNA clones which show homology to human  $\alpha_d$  encoding sequences. Example 25 describes animal models which resemble human disease states wherein modulation of  $\alpha_d$  is assayed for therapeutic capabilities.

#### Example 1

##### Attempt to Detect a Human Homolog of Canine $\alpha_{TM1}$

The monoclonal antibody Ca11.8H2 [Moore, et al., *supra*] specific for canine  $\alpha_{TM1}$  was tested for cross-reactivity on human peripheral blood leukocytes in an attempt to identify a human homolog of canine  $\alpha_{TM1}$ . Cell preparations (typically  $1 \times 10^6$  cells) were incubated with undiluted hybridoma supernatant or a purified mouse IgG-negative control antibody (10  $\mu$ g/ml) on ice in the presence of 0.1% sodium azide. Monoclonal antibody binding was detected by subsequent incubation with FITC-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) at 6  $\mu$ g/ml. Stained cells were fixed with 2% w/v paraformaldehyde in phosphate buffered saline (PBS) and were analyzed with a Facstar Plus fluorescence-activated cell sorter (Becton Dickinson, Mountain View, CA). Typically, 10,000 cells were analyzed using logarithmic amplification for fluorescence intensity.

The results indicated that Ca11.8H2 did not cross-react with surface proteins expressed on human peripheral blood leukocytes, while the control cells,

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neoplastic canine peripheral blood lymphocytes, were essentially all positive for  $\alpha_{TM1}$ .

Because the monoclonal antibody Ca11.8H2 specific for the canine  $\alpha$  subunit did not cross react with a human homolog, isolation of canine  $\alpha_{TM1}$  DNA was deemed a necessary prerequisite to isolate a counterpart human gene if one existed.

### Example 2

#### Affinity Purification Of Canine $\alpha_{TM1}$ For N-Terminal Sequencing

Canine  $\alpha_{TM1}$  was affinity purified in order to determine N-terminal amino acid sequences for oligonucleotide probe/primer design. Briefly, anti- $\alpha_{TM1}$  monoclonal antibody Ca11.8H2 was coupled to Affigel 10 chromatographic resin (BioRad, Hercules, CA) and protein was isolated by specific antibody-protein interaction. Antibody was conjugated to the resin, according to the BioRad suggested protocol, at a concentration of approximately 5 mg antibody per ml of resin. Following the conjugation reaction, excess antibody was removed and the resin blocked with three volumes of 0.1 M ethanolamine. The resin was then washed with thirty column volumes of phosphate buffered saline (PBS).

Twenty-five grams of a single dog spleen were homogenized in 250 ml of buffer containing 0.32 M sucrose in 25 mM Tris-HCl, Ph 8.0, with protease inhibitors. Nuclei and cellular debris were pelleted with centrifugation at 1000 g for 15 minutes. Membranes were pelleted from the supernatant with centrifugation at 100,000 g for 30 minutes. The membrane pellet was resuspended in 200 ml lysis buffer (50 mM NaCl, 50 mM borate, pH 8.0, with 2% NP-40) and incubated for 1 hour on ice. Insoluble material was then pelleted by centrifugation at 100,000 g for 60 minutes. Ten milliliters of the cleared lysate were transferred to a 15 ml polypropylene tube with 0.5 ml Ca11.8H2-conjugated Affigel 10 resin described above. The tube was incubated overnight at 4°C with rotation and the resin subsequently washed with 50 column volumes

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D-PBS. The resin was then transferred to a microfuge tube and boiled for ten minutes in 1 ml Laemmli (non-reducing) sample buffer containing 0.1 M Tris-HCl, pH 6.8, 2% SDS, 20% glycerol and 0.002% bromophenol blue. The resin was pelleted by centrifugation and discarded; the supernatant was treated with  
5 1/15 volume  $\beta$ -mercaptoethanol (Sigma, St. Louis, MO) and run on a 7% polyacrylamide gel. The separated proteins were transferred to Immobilon PVDF membrane (Millipore, Bedford, MA) as follows.

The gels were washed once in deionized, Millipore-filtered water and equilibrated for 15-45 minutes in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) transfer buffer, pH 10.5, with 10% methanol.  
10 Immobilon membranes were moistened with methanol, rinsed with filtered water, and equilibrated for 15-30 minutes in CAPS transfer buffer. The initial transfer was carried out using a Biorad transfer apparatus at 70 volts for 3 hours. The Immobilon membrane was removed after transfer and stained in filtered 0.1%  
15 R250 Coomassie stain for 10 minutes. Membranes were destained in 50% methanol/10% acetic acid three times, ten minutes each time. After destaining, the membranes were washed in filtered water and air-dried.

Protein bands of approximately 150 kD, 95 kD, 50 kD and 30 kD were detected. Presumably the 50 kD and 30 kD bands resulted from antibody  
20 contamination. N-terminal sequencing was then attempted on both the 150 kD and 95 kD bands, but the 95 kD protein was blocked, preventing sequencing. The protein band of 150 kD was excised from the membrane and directly sequenced with an Applied Biosystems (Foster City, CA) Model 473A protein sequencer according to the manufacturer's instructions. The resulting amino acid  
25 sequence is set in SEQ ID NO: 5 using single letter amino acid designations.

FNLDVEEPMVFQ

(SEQ ID NO: 5)

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The identified sequence included the FNLD sequence characteristic of  $\alpha$  subunits of the integrin family [Tamura, *et al.*, *J. Cell. Biol.* 111:1593-1604 (1990)].

Primer Design and Attempt to Amplify Canine  $\alpha_{TM1}$  Sequences

From the N-terminal sequence information, three oligonucleotide probes were designed for hybridization: a) "Tommer," a fully degenerate oligonucleotide; b) "Patmer," a partially degenerate oligonucleotide; and c) "Guessmer," a nondegenerate oligonucleotide based on mammalian codon usage. These probes are set out below as SEQ ID NOS: 6, 7 and 8, respectively. Nucleic acid symbols are in accordance with 37 C.F.R. §1.882 for these and all other nucleotide sequences herein.

5'-TTYAAYYTGGAYGTNGARGARCCNATGGTNTTYCA-3' (SEQ ID NO: 6)

5'-TTCAACCTGGACGTGGAGGAGCCCATGGTGTTCCTCAA-3' (SEQ ID NO: 7)

5'-TTCAACCTGGACGTNGAASANCCCATGGTCTTCCAA-3' (SEQ ID NO: 8)

Based on sequencing data, no relevant clones were detected using these oligonucleotides in several low stringency hybridizations to a canine spleen/peripheral blood macrophage cDNA library cloned into  $\lambda$ ZAP (Stratagene, La Jolla, CA).

Four other oligonucleotide primers, designated 5'Deg, 5'Spec, 3'Deg and 3'Spec (as set out in SEQ ID NOS: 9, 10, 11 and 12, respectively, wherein Deg indicates degenerate and Spec indicates non-degenerate) were subsequently designed based on the deduced N-terminal sequence for attempts to amplify canine  $\alpha_{TM1}$  sequences by PCR from phage library DNA purified from plate lysates of the Stratagene library described above.

5'-TTYAAYYTNGAYGTNGARGARCC-3' (SEQ ID NO: 9)

5'-TTYAAYYTGGACGTNGAAGA-3' (SEQ ID NO: 10)

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5'-TGRAANACCATNGGYTC-3' (SEQ ID NO: 11)

5'-TTGGAAGACCATNGGYTC-3' (SEQ ID NO: 12)

5 The  $\alpha_{TM1}$  oligonucleotide primers were paired with T3 or T7 vector primers, as set out in SEQ ID NOS: 13 and 14, respectively, which hybridize to sequences flanking the polylinker region in the Bluescript phagemid found in  $\lambda$ ZAP.

5'-ATTAACCCTCACTAAAG-3' (SEQ ID NO: 13)

5'-AATACGACTCACTATAG-3' (SEQ ID NO: 14)

10 The PCR amplification was carried out in *Taq* buffer (Boehringer Mannheim, Indianapolis, IN) containing magnesium with 150 ng of library DNA, 1  $\mu$ g of each primer, 200  $\mu$ M dNTPs and 2.5 units *Taq* polymerase (Boehringer Mannheim) and the products were separated by electrophoresis on a 1% agarose gel in Tris-Acetate-EDTA (TAE) buffer with 0.25  $\mu$ g/ml ethidium bromide. DNA was transferred to a Hybond (Amersham, Arlington Heights, IL) membrane by wicking overnight in 10X SSPE. After transfer, the immobilized DNA was  
15 denatured with 0.5 M NaOH with 0.6 M NaCl, neutralized with 1.0 M Tris-HCl, pH 8.0, in 1.5 M NaCl, and washed with 2X SSPE before UV crosslinking with a Stratalinker (Stratagene) crosslinking apparatus. The membrane was incubated in prehybridization buffer (5X SSPE, 4X Denhardts, 0.8% SDS, 30% formamide)  
20 for 2 hr at 50°C with agitation.

Oligonucleotide probes 5'Deg, 5'Spec, 3'Deg and 3'Spec (SEQ ID NOS: 9, 10, 11 and 12, respectively) were labeled using a Boehringer Mannheim kinase buffer with 100-300  $\mu$ Ci  $\gamma$ P<sup>32</sup>-dATP and 1-3 units of polynucleotide kinase for 1-3 hr at 37°C. Unincorporated label was removed with  
25 Sephadex G-25 fine (Pharmacia, Piscataway, NJ) chromatography using 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (TE) buffer and the flow-through added directly to the prehybridization solution. Membranes were probed for 16 hr at 42°C with

agitation and washed repeatedly, with a final stringency wash of 1X SSPE/0.1% SDS at 50° for 15 min. The blot was then exposed to Kodak X-Omat AR film for 1-4 hours at -80°C.

The oligonucleotides 5'Deg, 5'Spec, 3'Deg and 3'Spec only hybridized to PCR products from the reactions in which they were used as primers and failed to hybridize as expected to PCR products from the reactions in which they were not used as primers. Thus, it was concluded that none of the PCR products were specific for  $\alpha_{TM1}$  because no product hybridized with all of the appropriate probes.

### Example 3

### Large Scale Affinity Purification Of Canine $\alpha_{TM1}$ For Internal Sequencing

In order to provide additional amino acid sequence for primer design, canine  $\alpha_{TM1}$  was purified for internal sequencing. Three sections of frozen spleen (approximately 50 g each) and frozen cells from two partial spleens from adult dogs were used to generate protein for internal sequencing. Fifty grams of spleen were homogenized in 200-300 ml borate buffer with a Waring blender. The homogenized material was diluted with 1 volume of buffer containing 4% NP-40; and the mixture then gently agitated for at least one hour. The resulting lysate was cleared of large debris by centrifugation at 2000 g for 20 min, and then filtered through either a Corning (Corning, NY) prefilter or a Corning 0.8 micron filter. The lysate was further clarified by filtration through the Corning 0.4 micron filter system.

25 Splenic lysate and the antibody-conjugated Affigel 10 resin described in Example 2 were combined at a 150:1 volume ratio in 100 ml aliquots and incubated overnight at 4°C with rocking. The lysate was removed after centrifugation at 1000 g for 5 minutes, combined with more antibody-conjugated Affigel 10 resin and incubated overnight as above. The absorbed resin aliquots were then combined and washed with 50 volumes D-PBS/0.1% Tween 20 and the

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resin transferred to a 50 ml Biorad column. Adsorbed protein was eluted from the resin with 3-5 volumes of 0.1 M glycine (pH 2.5); fractions of approximately 900  $\mu$ l were collected and neutralized with 100  $\mu$ l 1 M Tris buffer, pH 8.0. Aliquots of 15  $\mu$ l were removed from each fraction and boiled in an equal volume of 2X Laemmli sample buffer with 1/15 volume 1 M dithiothreitol (DTT). These samples were electrophoresed on 8% Novex (San Diego, CA) polyacrylamide gels and visualized either by Coomassie stain or by silver stain using a Daiichi kit (Enprotech, Natick, MA) according to the manufacturer's suggested protocol. Fractions which contained the largest amounts of protein were combined and concentrated by vacuum. The remaining solution was diluted by 50% with reducing Laemmli sample buffer and run on 1.5 mm 7% polyacrylamide gels in Tris-glycine/SDS buffer. Protein was transferred from the gels to Immobilon membrane by the procedure described in Example 2 using the Hoefer transfer apparatus.

The protein bands corresponding to canine  $\alpha_{TM1}$  were excised from 10 PVDF membranes and resulted in approximately 47  $\mu$ g total protein. The bands were destained in 4 ml 50% methanol for 5 minutes, air dried and cut into 1 x 2 mm pieces. The membrane pieces were submerged in 2 ml 95% acetone at 4°C for 30 minutes with occasional vortexing and then air dried.

Prior to proteolytic cleavage of the membrane bound protein, 3 mg of cyanogen bromide (CNBr) (Pierce, Rockford, IL) were dissolved in 1.25 ml 70% formic acid. This solution was then added to a tube containing the PVDF membrane pieces and the tube incubated in the dark at room temperature for 24 hours. The supernatant (S1) was then removed to another tube and the membrane pieces washed with 0.25 ml 70% formic acid. This supernatant (S2) was removed and added to the previous supernatant (S1). Two milliliters of Milli Q water were added to the combined supernatants (S1 and S2) and the solution lyophilized. The PVDF membrane pieces were dried under nitrogen and extracted again with 1.25 ml 60% acetonitrile, 0.1% tetrafluoroacetic acid (TFA) at 42°C for 17 hours.



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This supernatant (S3) was removed and the membrane pieces extracted again with 1.0 ml 80% acetonitrile with 0.08% TFA at 42°C for 1 hour. This supernatant (S4) was combined with the previous supernatants (S1, S2 and S3) and vacuum dried.

5                   The dried CNBr fragments were then dissolved in 63  $\mu$ l 8 M urea, 0.4 M  $\text{NH}_4\text{HCO}_3$ . The fragments were reduced in 5  $\mu$ l 45 mM dithiothreitol (DTT) and subsequently incubated at 50°C for 15 minutes. The solution was then cooled to room temperature and the fragments alkylated by adding 5  $\mu$ l 100 mM iodoacetamide (Sigma, St. Louis, MO). Following a 15 minute incubation at  
10                   room temperature, the sample was diluted with 187  $\mu$ l Milli Q water to a final urea concentration of 2.0 M. Trypsin (Worthington, Freehold, NJ) was then added at a ratio of 1:25 (w:w) of enzyme to protein and the protein digested for 24 hours at 37°C. Digestion was terminated with addition of 30  $\mu$ l TFA.

                  The protein fragments were then separated with high performance  
15                   liquid chromatography (HPLC) on a Waters 625 LC system (Millipore, Milford, MA) using a 2.1 x 250 mm, 5 micron Vydac C-18 column (Vydac, Hesperia, CA) equilibrated in 0.05% TFA and HPLC water (buffer A). The peptides were eluted with increasing concentration of 80% acetonitrile in 0.04% TFA (buffer B) with a gradient of 38-75% buffer B for 65-95 minutes and 75-98% buffer B for  
20                   95-105 minutes. Peptides were fractionated at a flow rate of 0.2 ml/minute and detected at 210 nm.

                  Following fractionation, the amino acid sequence of the peptides was analyzed by automated Edman degradation performed on an Applied Biosystems Model 437A protein sequencer using the manufacturer's standard  
25                   cycles and the Model 610A Data Analysis software program, Version 1.2.1. All sequencing reagents were supplied by Applied Biosystems. The amino acid sequences of seven of the eight internal fragments are set out below wherein "X" indicates the identity of the amino acid was not certain.

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VFQEXGAGFGQ (SEQ ID NO: 15)  
 LYDXVAATGLXQPI (SEQ ID NO: 16)  
 PLEYXDVIPQAE (SEQ ID NO: 17)  
 FQEGFSXVLX (SEQ ID NO: 18)  
 5 TSPTFIXMSQENVD (SEQ ID NO: 19)  
 LVVGAPLEVVA VXQTGR (SEQ ID NO: 20)  
 LDXXPXDTA (SEQ ID NO: 21)

Primer Design

One internal amino acid sequence (set out in SEQ ID NO: 22)  
 10 obtained was then used to design a fully degenerate oligonucleotide primer,  
 designated p4(R) as set out in SEQ ID NO: 23.

FGEQFSE (SEQ ID NO: 22)  
 5'-RAANCCYTCYTGRAAACTYTC-3' (SEQ ID NO: 23)

Example 415 PCR Cloning Of A Canine  $\alpha_{TM1}$  Fragment

The 5' portion of the canine  $\alpha_{TM1}$  gene was amplified from  
 double-stranded canine splenic cDNA by PCR.

A. Generation of Double Stranded Canine Spleen cDNA

One gram of frozen material from a juvenile dog spleen was ground  
 20 in liquid nitrogen on dry ice and homogenized in 20 ml RNA-Stat 60 buffer (Tel-  
 Test B, Inc, Friendswood, TX). Four ml chloroform were added, and the  
 solution extracted by centrifugation at 12,000 g for 15 minutes. RNA was  
 precipitated from the aqueous layer with 10 ml ethanol. Poly A<sup>+</sup> RNA was then  
 selected on Dynal Oligo dT Dynabeads (Dynal, Oslo, Norway). Five aliquots of  
 25 100  $\mu$ g total RNA were combined and diluted with an equal volume of 2X binding

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buffer (20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 1 mM EDTA, 0.1% SDS). RNA was then incubated 5 minutes with the Oligo dT Dynabeads (1.0 ml or 5 mg beads for all the samples). Beads were washed with buffer containing 10 mM Tris-HCl, pH 7.5, 0.15 M LiCl, 1 mM EDTA and 0.1% SDS, according to the manufacturer's suggested protocol prior to elution of poly A<sup>+</sup> mRNA with 2 mM EDTA, pH 7.5. Double-stranded cDNA was then generated using the eluted poly A<sup>+</sup> mRNA and the Boehringer Mannheim cDNA Synthesis Kit according to the manufacturer's suggested protocol.

#### B. Isolation of a Partial Canine $\alpha_{TM1}$ cDNA

Oligonucleotide primers 5'Deg (SEQ ID NO: 9) and p4(R) (SEQ ID NO: 23) were employed in a standard PCR reaction using 150 ng double-stranded cDNA, 500 ng of each primer, 200  $\mu$ M dNTPs and 1.5 units *Taq* polymerase (Boehringer Mannheim) in *Taq* buffer (Boehringer Mannheim) with magnesium. The resulting products (1  $\mu$ l of the original reaction) were subjected to a second round of PCR with the same primers to increase product yield. This band was eluted from a 1% agarose gel onto Schleicher & Schuell (Keene, NH) NA45 paper in a buffer containing 10 mM Tris-HCl, pH 8, 1 mM EDTA, 1.5 M NaCl at 65°C, precipitated, and ligated into the pCR<sup>™</sup>II vector (Invitrogen, San Diego, CA) using the TA cloning kit (Invitrogen) and the manufacturer's suggested protocol. The ligation mixture was transformed by electroporation into XL-1 Blue bacteria (Stratagene). One clone, 2.7, was determined to contain sequences corresponding to  $\alpha_{TM1}$  peptide sequences which were not utilized in design of the primers.

Sequencing was performed with an Applied Biosystems 373A DNA sequencer (Foster City, CA) with a Dye-deoxy terminator cycle sequence kit (ABI) in which fluorescent-labeled dNTPs were incorporated in an asymmetric PCR reaction [McCabe, "Production of Single Stranded DNA by Asymmetric PCR," in PCR Protocols: A Guide to Methods and Applications, Innis, *et al.*

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(eds.) pp. 76-83 Academic Press: New York (1990)] as follows. Samples were held at 96°C for 4 minutes and subjected to 25 cycles of the step sequence: 96°C, for 15 seconds; 50°C for 1 second; 60°C for 4 minutes. Sequence data was automatically down-loaded into sample files on the computer that included chromatogram and text files. The sequence of the entire insert of clone 2.7 is set out in SEQ ID NO: 24.

Attempts to isolate the full length canine  $\alpha_{TM1}$  cDNA from the Stratagene library (as described in Example 2) were unsuccessful. Approximately  $1 \times 10^6$  phage plaques were screened by hybridization under low stringency conditions using 30% formamide with clone 2.7 as a probe, but no positive clones resulted. Attempts to amplify relevant sequences downstream from those represented in clone 2.7 using specific oligonucleotides derived from clone 2.7 or degenerate primers based on amino acid sequence from other peptide fragments paired with a degenerate oligonucleotide based on the conserved  $\alpha$  subunit amino acid motif GFFKR [Tamura, *et al.*, *supra*] were also unsuccessful.

#### Example 5

##### Cloning Of A Putative Human Homolog Of Canine $\alpha_{TM1}$

To attempt the isolation of a human sequence homologous to canine  $\alpha_{TM1}$  the approximately 1 kb canine  $\alpha_{TM1}$  fragment from clone 2.7 was used as a probe. The probe was generated by PCR under conditions described in Example 2 using NT2 (as set out in SEQ ID NO: 25) and p4(R) (SEQ ID NO: 23) primers.

5'-GTNTTYCARGARGAYGG-3'

(SEQ ID NO: 25)

The PCR product was purified using the Qiagen (Chatsworth, GA) Quick Spin kit and the manufacturer's suggested protocol. The purified DNA (200 ng) was labeled with 200  $\mu$ Ci  $\alpha^{32}$ PdCTP using the Boehringer Mannheim Random Prime

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Labelling kit and the manufacturer's suggested protocol. Unincorporated isotope was removed with Sephadex G25 (fine) gravity chromatography. The probe was denatured with 0.2 N NaOH and neutralized with 0.4 M Tris-HCl, pH 8.0, before use.

5 Colony lifts on Hybond filters (Amersham) of a human spleen cDNA library in pCDNA/Amp (Invitrogen, San Diego, CA) were prepared. The filters were initially denatured and neutralized as described in Example 2 and subsequently incubated in a prehybridization solution (8 ml/filter) with 30% formamide at 50°C with gentle agitation for 2 hours. Labeled probe as described  
10 above was added to this solution and incubated with the filters for 14 hours at 42°C. The filters were washed twice in 2X SSC/0.1% SDS at 37°C and twice in 2X SSC/0.1% SDS at 50°C. Final stringency washes were 1X SSC/0.1% SDS, twice at 65°C (1X SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0). Filters were exposed to Kodak X-Omat AR film for six hours with an intensifying  
15 screen. Colonies giving signals on duplicate lifts were streaked on LB medium with magnesium (LBM)/carbenicillin plates and incubated overnight at 37°C. Resulting streaked colonies were lifted with Hybond filters and these filters were treated as above. The filters were hybridized under more stringent conditions with the 1 kb probe from clone 2.7, labeled as previously described, in a 50%  
20 formamide hybridization solution at 50°C for 3 hours. Probed filters were washed with a final stringency of 0.1 X SSC/0.1% SDS at 65°C and exposed to Kodak X-Omat AR film for 2.5 hours at -80°C with an intensifying screen. Positive colonies were identified and cultured in LBM/carbenicillin medium overnight. DNA from the cultures was prepared using the Promega Wizard  
25 miniprep kit according to the manufacturer's suggested protocol and the resulting DNA was sequenced.

The initial screening resulted in 18 positive clones, while the secondary screening under more stringent hybridization conditions produced one positive clone which was designated 19A2. The DNA and deduced amino acid

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sequences of the human  $\alpha_d$  clone 19A2 are set out in SEQ ID NOS: 1 and 2, respectively.

#### Characteristics Of The Human $\alpha_d$ cDNA and Predicted Polypeptide

5 Clone 19A2 encompasses the entire coding region for the mature protein, plus 48 bases (16 amino acid residues) of the 5' upstream signal sequence and 241 bases of 3' untranslated sequence which do not terminate in a polyadenylation sequence. The core molecular weight of the mature protein is predicted to be around 125 kD. The extracellular domain is predicted to encompass approximately amino acid residues 17 through 1108 of SEQ ID NO:

10 2. This extracellular region is contiguous with about a 20 amino acid region homologous to the human CD11c transmembrane region (residues 1109 through 1128 of SEQ ID NO: 2). The cytoplasmic domain comprises approximately 30 amino acids (about residues 1129 through 1161 of SEQ ID NO: 2). The protein also contains a region (around residues 150 through 352) of approximately 202

15 amino acids homologous to the I (insertion) domain common to CD11a, CD11b and CD11c [Larson and Springer, *supra*],  $\alpha_E$  [Shaw, *et al.*, *J.Biol.Chem.* 269:6016-6025 (1994)] and in VLA-1 and VLA-2, [Tamura, *et al.*, *supra*]. The I domain in other integrins has been shown to participate in ICAM binding [Landis, *et al.*, *J.Cell.Biol.* 120:1519-1527 (1993); Diamond, *et al.*, *J.Cell.Biol.*

20 120:1031-1043 (1993)], suggesting that  $\alpha_d$  may also bind members of the ICAM family of surface molecules. This region has not been demonstrated to exist in any other integrin subunits.

The deduced amino acid sequence of  $\alpha_d$  shows approximately 36% identity to that of CD11a, approximately 60% identity to CD11b and

25 approximately 66% identity to CD11c. An alignment of amino acid sequences for (CD11b SEQ ID NO: 3), CD11c (SEQ ID NO: 4) and  $\alpha_d$  (SEQ ID NO: 2) is presented in Figure 1.

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The cytoplasmic domains of  $\alpha$  subunits in  $\beta_2$  integrins are typically distinct from one another within the same species, while individual  $\alpha$  subunits show high degrees of homology across species boundaries. Consistent with these observations, the cytoplasmic region of  $\alpha_d$  differs markedly from CD11a, CD11b, and CD11c except for a membrane proximal GFFKR amino acid sequence which has been shown to be conserved among all  $\alpha$  integrins [Rojiani, *et al.*, *Biochemistry* 30: 9859-9866 (1991)]. Since the cytoplasmic tail region of integrins has been implicated in "inside out" signaling and in avidity regulation [Landis *et al.*, *supra*], it is possible that  $\alpha_d$  interacts with cytosolic molecules distinct from those interacting with CD11a, CD11b, and CD11c, and, as a result, participates in signaling pathways distinct from those involving other  $\beta_2$  integrins.

The extracellular domain of  $\alpha_d$  contains a conserved DGSGS amino acid sequence adjacent the I-domain; in CD11b, the DGSGS sequence is a metal-binding region required for ligand interaction [Michishita, *et al.* *Cell* 72:857-867 (1993)]. Three additional putative cation binding sites in CD11b and CD11c are conserved in the  $\alpha_d$  sequence at amino acids 465-474, 518-527, and 592-600 in clone 19A2 (SEQ ID NO: 1). The  $\alpha_d$  I-domain is 36%, 62%, and 57% identical to the corresponding regions in CD11a, CD11b, and CD11c, respectively, and the relatively low sequence homology in this region suggests that  $\alpha_d$  may interact with a set of extracellular proteins distinct from proteins with which other known  $\beta_2$  integrins interact. Alternatively, the affinity of  $\alpha_d$  for known  $\beta_2$  integrin ligands, for example, ICAM-1, ICAM-2 and/or ICAM-R, may be distinct from that demonstrated for the other  $\beta_2$  integrin/ICAM interactions. [See Example 12.]

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**Example 6****Northern Analysis of Human  $\alpha_d$  Expression in Tissues**

In order to determine the relative level of expression and tissue specificity of  $\alpha_d$ , Northern analysis was performed using fragments from clone 19A2 as probes. Approximately 10  $\mu$ g of total RNA from each of several human tissues or cultured cell lines were loaded on a formaldehyde agarose gel in the presence of 1  $\mu$ g of ethidium bromide. After electrophoresis at 100 V for 4 hr, the RNA was transferred to a nitrocellulose membrane (Schleicher & Schuell) by wicking in 10X SSC overnight. The membrane was baked 1.5 hr at 80°C under vacuum. Prehybridization solution containing 50% formamide in 3-(N-morpholino)propane sulfonic acid (MOPS) buffer was used to block the membrane for 3 hr at 42°C. Fragments of clone 19A2 were labeled with the Boehringer Mannheim Random Prime kit according to the manufacturer's instructions including both  $\alpha$ P<sup>32</sup>dCTP and  $\alpha$ P<sup>32</sup>dTTP. Unincorporated label was removed on a Sephadex G25 column in TE buffer. The membrane was probed with 1.5 x 10<sup>6</sup> counts per ml of prehybridization buffer. The blot was then washed successively with 2X SSC/0.1% SDS at room temperature, 2X SSC/0.1% SDS at 42°C, 2X SSC/0.1% SDS at 50°C, 1X SSC/0.1% SDS at 50°C, 0.5X SSC/0.1% SDS at 50°C and 0.1X SSC/0.1% SDS at 50°C. The blot was then exposed to film for 19 hr.

Hybridization using a BsrXI fragment from clone 19A2 (corresponding to nucleotides 2011 to 3388 in SEQ ID NO: 1) revealed a weak signal in the approximately 5 kb range in liver, placenta, thymus, and tonsil total RNA. No signal was detected in kidney, brain or heart samples. The amount of RNA present in the kidney lane was minimal, as determined with ethidium bromide staining.

When using a second fragment of clone 19A2 (encompassing the region from bases 500 to 2100 in SEQ ID NO: 1), RNA transcripts of two different sizes were detected in a human multi-tissue Northern (MTN) blot using



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polyA<sup>+</sup> RNA (Clontech). An approximately 6.5 kb band was observed in spleen and skeletal muscle, while a 4.5 kb band was detected in lung and peripheral blood leukocytes. The variation in sizes observed could be caused by tissue specific polyadenylation, cross reactivity of the probe with other integrin family members, or hybridization with alternatively spliced mRNAs.

Northern analysis using a third fragment from clone 19A2, spanning nucleotides 2000 to 3100 in SEQ ID NO: 1, gave results consistent with those using the other clone 19A2 fragments.

RNA from three myeloid lineage cell lines was also probed using the fragments corresponding to nucleotides 500 to 2100 and 2000 to 3100 in SEQ ID NO:1. A THP-1 cell line, previously stimulated with PMA, gave a diffuse signal in the same size range (approximately 5.0 kb), with a slightly stronger intensity than the tissue signals. RNA from unstimulated and DMSO-stimulated HL-60 cells hybridized with the  $\alpha_d$  probe at the same intensity as the tissue samples, however, PMA treatment seemed to increase the signal intensity. Since PMA and DMSO drive HL-60 cell differentiation toward monocyte/macrophage and granulocyte pathways, respectively, this result suggests enhanced  $\alpha_d$  expression in monocyte/macrophage cell types. U937 cells expressed the  $\alpha_d$  message and this signal did not increase with PMA stimulation. No band was detected in Molt, Daudi, H9, JY, or Jurkat cells.

### Example 7

#### Transient Expression of Human $\alpha_d$ Constructs

##### A. Generation of expression constructs

The human clone 19A2 lacks an initiating methionine codon and possibly some of the 5' signal sequence. Therefore, in order to generate a human expression plasmid containing 19A2 sequences, two different strategies were used. In the first, two plasmids were constructed in which signal peptide sequences derived from genes encoding either CD11b or CD11c were spliced into clone

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19A2 to generate a chimeric  $\alpha_d$  sequence. In the second approach, a third plasmid was constructed in which an adenosine base was added at position 0 in clone 19A2 to encode an initiating methionine.

5 The three plasmids contained different regions which encoded the 5' portion of the  $\alpha_d$  sequence or the chimeric  $\alpha_d$  sequence. The  $\alpha_d$  region was PCR amplified (see conditions in Example 2) with a specific 3' primer BamRev (set out below in SEQ ID NO: 26) and one of three 5' primers. The three 5' primers contained in sequence: (1) identical nonspecific bases at positions 1-6 allowing for digestion, an *EcoRI* site from positions 7-12 and a consensus Kozak  
10 sequence from positions 13-18; (2) a portion of the CD11b (primer ER1B) or CD11c (primer ER1C) signal sequence, or an adenosine (primer ER1D); and (3) an additional 15-17 bases specifically overlapping 5' sequences from clone 19A2 to allow primer annealing. Primers ER1B, ER1C or ER1D are set out in SEQ ID NOS: 27, 28 or 29, respectively, where the initiating methionine codon is  
15 underlined and the *EcoRI* site is double underlined.

5'-CCACTGTCAGGATGCCCCGTG-3' (SEQ ID NO: 26)

5'-AGTTACGAATTCGCCACCATGGCTCTACGGGTGCTT (SEQ ID NO: 27)

20 5'-AGTTACGAATTCGCCACCATGACTCGGACTGTGCTT (SEQ ID NO: 28)

5'-AGTTACGAATTCGCCACCATGACCTTCGGCACTGTG (SEQ ID NO: 29)

The resulting PCR product was digested with *EcoRI* and *BamHI*.

25 All three plasmids contained a common second  $\alpha_d$  region (to be inserted immediately downstream from the 5' region described in the previous paragraph) including the 3' end of the  $\alpha_d$  clone. The second  $\alpha_d$  region, which

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extended from nucleotide 625 into the *Xba*I site in the vector 3' polylinker region of clone 19A2, was isolated by digestion of clone 19A2 with *Bam*HI and *Xba*I.

Three ligation reactions were prepared in which the 3'  $\alpha_d$  *Bam*HI/*Xba*I fragment was ligated to one of the three 5'  $\alpha_d$  *Eco*RI/*Bam*HI fragments using Boehringer Mannheim ligase buffer and T4 ligase (1 unit per reaction). After a 4 hour incubation at 14°C, an appropriate amount of vector pcDNA.3 (Invitrogen) digested with *Eco*RI and *Xba*I was added to each reaction with an additional unit of ligase. Reactions were allowed to continue for another 14 hours. One tenth of the reaction mixture was then transformed into competent XL-1 Blue cells. The resulting colonies were cultured and the DNA isolated as in Example 5. Digestion with *Eco*RI identified three clones which were positive for that restriction site, and thus, the engineered signal sequences. The clones were designated pATM.B1 (CD11b/ $\alpha_d$ , from primer ER1B), pATM.C10 (CD11c/ $\alpha_d$ , from primer ER1C) and pATM.D12 (adenosine/ $\alpha_d$  from primer ER1d). The presence of the appropriate signal sequences in each clone was verified by nucleic acid sequencing.

#### B. Transfection of COS Cells

Expression from the  $\alpha_d$  plasmids discussed above was effected by cotransfection of COS cells with the individual plasmids and a CD18 expression plasmid, pRC.CD18. As a positive control, COS cells were also co-transfected with the plasmid pRC.CD18 and a CD11a expression plasmid, pDC.CD11A.

Cells were passaged in culture medium (DMEM/10%FBS/pen-strep) into 10 cm Corning tissue culture-treated petri dishes at 50% confluency 16 hours prior to transfection. Cells were removed from the plates with Versene buffer (0.5 mM NaEDTA in PBS) without trypsin for all procedures. Before transfection, the plates were washed once with serum-free DMEM. Fifteen micrograms of each plasmid were added to 5 ml transfection buffer (DMEM with 20  $\mu$ g/ml DEAE-Dextran and 0.5 mM chloroquine) on each plate. After 1.5

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hours incubation at 37°C, the cells were shocked for 1 minute with 5 ml DMEM/10% DMSO. This DMSO solution was then replaced with 10 ml/plate culture medium.

Resulting transfectants were analyzed by ELISA, FACS, and immunoprecipitation as described in Examples 8, 9, and 10.

### Example 8

#### ELISA Analysis of COS Transfectants

In order to determine if the COS cells co-transfected with CD18 expression plasmid pRC.CD18 and an  $\alpha_d$  plasmid expressed  $\alpha_d$  on the cell surface in association with CD18, ELISAs were performed using primary antibodies raised against CD18 (e.g., TS1/18 purified from ATCC HB203). As a positive control, ELISAs were also performed on cells co-transfected with the CD18 expression plasmid and a CD11a expression plasmid, pDC.CD11A. The primary antibodies in this control included CD18 antibodies and anti-CD11a antibodies (e.g., TS1/22 purified from ATCC HB202).

For ELISA, cells from each plate were removed with Versene buffer and transferred to a single 96-well flat-bottomed Corning tissue culture plate. Cells were allowed to incubate in culture media 2 days prior to assay. The plates were then washed twice with 150  $\mu$ l/well D-PBS/0.5% teleost skin gelatin (Sigma) solution. This buffer was used in all steps except during the development. All washes and incubations were performed at room temperature. The wells were blocked with gelatin solution for 1 hour. Primary antibodies were diluted to 10  $\mu$ g/ml in gelatin solution and 50  $\mu$ l were then added to each well. Triplicate wells were set up for each primary antibody. After 1 hour incubation, plates were washed 3X with 150  $\mu$ l/well gelatin solution. Secondary antibody (goat anti-mouse Ig/HRP-Fc specific [Jackson, West Grove, PA]) at a 1:3500 dilution was added at 50  $\mu$ l/well and plates were incubated for 1 hour. After three washes, plates were developed for 20 minutes with 100  $\mu$ l/well o-

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phenyldiamine (OPD) (Sigma) solution (1 mg/ml OPD in citrate buffer) before addition of 50  $\mu$ l/well 15% sulfuric acid.

Analysis of transfectants in the ELISA format with anti-CD18 specific antibodies revealed no significant expression above background in cells transfected only with the plasmid encoding CD18. Cells co-transfected with plasmid containing CD11a and CD18 showed an increase in expression over background when analyzed with CD18 specific antibodies or with reagents specific for CD11a. Further analysis of cells co-transfected with plasmids encoding CD18 and one of the  $\alpha_d$  expression constructs (pATM.C10 or pATM.D12) revealed that cell surface expression of CD18 was rescued by concomitant expression of  $\alpha_d$ . The increase in detectable CD18 expression in COS cells transfected with pATM.C10 or pATM.D12 was comparable to that observed in co-transfected CD11a/CD18 positive control cells.

#### Example 9

##### FACS Analysis of COS Transfectants

For FACS analysis, cells in petri dishes were fed with fresh culture medium the day after transfection and allowed to incubate 2 days prior to the assay. Transfectant cells were removed from the plates with 3 ml Versene, washed once with 5 ml FACS buffer (DMEM/2% FBS/0.2% sodium azide) and diluted to 500,000 cells/sample in 0.1 ml FACS buffer. Ten microliters of either 1 mg/ml FITC-conjugated CD18, CD11a, or CD11b specific antibodies (Becton Dickinson) or 800  $\mu$ g/ml CFSE-conjugated murine 23F2G (anti-CD18) (ATCC HB11081) were added to each sample. Samples were then incubated on ice for 45 minutes, washed 3X with 5 ml/wash FACS buffer and resuspended in 0.2 ml FACS buffer. Samples were processed on a Becton Dickinson FACscan and the data analyzed using Lysys II software (Becton Dickinson).

COS cells transfected with CD18 sequences only did not stain for CD18, CD11a or CD11b. When co-transfected with CD11a/CD18, about 15%

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of the cells stained with antibodies to CD11a or CD18. All cells transfected with CD18 and any  $\alpha_d$  construct resulted in no detectable staining for CD11a and CD11b. The pATM.B1, pATM.C10 and pATM.D12 groups stained 4%, 13% and 8% positive for CD18, respectively. Fluorescence of the positive population in the CD11a/CD18 group was 4-fold higher than background. In comparison, the co-transfection of  $\alpha_d$  constructs with the CD18 construct produced a positive population that showed a 4- to 7-fold increase in fluorescence intensity over background.

#### Example 10

##### 10     Biotin-Labeled Immunoprecipitation of          Human $\alpha_d$ /CD18 Complexes from Co-transfected COS Cells

Immunoprecipitation was attempted on cells co-transfected with CD18 and each of the  $\alpha_d$  expression plasmids separately described in Example 7 in order to determine if  $\alpha_d$  could be isolated as part of the  $\alpha\beta$  heterodimer complex characteristic of integrins.

15     Transfected cells ( $1-3 \times 10^8$  cells/group) were removed from petri dishes with Versene buffer and washed 3 times in 50 ml/group D-PBS. Each sample was labeled with 2 mg Sulpho-NHS Biotin (Pierce, Rockford, IL) for 15 minutes at room temperature. The reaction was quenched by washing 3 times in 20     50 ml/sample cold D-PBS. Washed cells were resuspended in 1 ml lysis buffer (1% NP40, 50 mM Tris-HCl, pH 8.0, 0.2 M NaCl, 2 mM  $\text{Ca}^{++}$ , 2 mM  $\text{Mg}^{++}$ , and protease inhibitors) and incubated 15 minutes on ice. Insoluble material was pelleted by centrifugation at 10,000 g for 5 minutes, and the supernatant removed to fresh tubes. In order to remove material non-specifically reactive with mouse 25     immunoglobulin, a pre-clearance step was initially performed. Twenty-five micrograms of mouse immunoglobulin (Cappel, West Chester, PA) was incubated with supernatants at 4°C. After 2.5 hr, 100  $\mu\text{l}$  (25  $\mu\text{g}$ ) rabbit anti-mouse Ig conjugated Sepharose (prepared from Protein A Sepharose 4B and rabbit anti-mouse IgG, both from Zymed, San Francisco, CA) was added to each sample;

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incubation was continued at 4°C with rocking for 16 hours. Sepharose beads were removed from the supernatants by centrifugation. After pre-clearance, the supernatants were then treated with 20 µg anti-CD18 antibody (TS1.18) for 2 hours at 4°C. Antibody/antigen complexes were isolated from supernatants by incubation with 100 µl/sample rabbit anti-mouse/Protein A-sepharose preparation described above. Beads were washed 4 times with 10 mM HEPES, 0.2 M NaCl, and 1% Triton-X 100. Washed beads were pelleted and boiled for 10 minutes in 20 µl 2X Laemmli sample buffer with 2% β-mercaptoethanol. Samples were centrifuged and run on an 8% pre-poured Novex polyacrylamide gel (Novex) at 100 V for 30 minutes. Protein was transferred to nitrocellulose membranes (Schleicher & Schuell) in TBS-T buffer at 200 mAmps for 1 hour. Membranes were blocked for 2 hr with 3% BSA in TBS-T. Membranes were treated with 1:6000 dilution of Strep-avidin horse radish peroxidase (POD) (Boehringer Mannheim) for 1 hour, followed by 3 washes in TBS-T. The Amersham Enhanced Chemiluminescence kit was then used according to the manufacturer's instructions to develop the blot. The membrane was exposed to Hyperfilm MP (Amersham) for 0.5 to 2 minutes.

Immunoprecipitation of CD18 complexes from cells transfected with pRC.CD18 and either pATM.B1, pATM.C10 or pATM.D12 revealed surface expression of a heterodimeric species consisting of approximately 100 kD β chain, consistent with the predicted size of CD18, and an α chain of approximately 150 kD, corresponding to α<sub>d</sub>.

### Example 11

#### Stable Transfection of Human α<sub>d</sub> in Chinese Hamster Ovary Cells

To determine whether α<sub>d</sub> is expressed on the cell surface as a heterodimer in association with CD18, cDNAs encoding each chain were both transiently and stably transfected into a cell line lacking both α<sub>d</sub> and CD18.

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For these experiments,  $\alpha_d$  cDNA was augmented with additional leader sequences and a Kozak consensus sequence, as described in Example 7, and subcloned into expression vector pcDNA3. The final construct, designated pATM.D12, was co-transfected with a modified commercial vector, pDC1.CD18 encoding human CD18 into dihydrofolate reductase (DHFR)<sup>-</sup> Chinese hamster ovary (CHO) cells. The plasmid pDC1.CD18 encodes a DHFR<sup>+</sup> marker and transfectants can be selected using an appropriate nucleoside-deficient medium. The modifications which resulted in pDC1.CD18 are as follows.

The plasmid pRC/CMV (Invitrogen) is a mammalian expression vector with a cytomegalovirus promoter and ampicillin resistance marker gene. A DHFR gene from the plasmid pSC1190-DHFR was inserted into pRC/CMV 5' of the SV40 origin of replication. In addition, a polylinker from the 5' region of the plasmid pHF2G-DHF was ligated into the pRC/CMV/DHFR construct, 3' to the DHFR gene. CD18 encoding sequences are subsequently cloned into the resulting plasmid between the 5' flanking polylinker region and the bovine growth hormone poly A encoding region.

Surface expression of CD18 was analyzed by flow cytometry using the monoclonal antibody TS1/18. Heterodimer formation detected between  $\alpha_d$  and CD18 in this cell line was consistent with the immunoprecipitation described in Example 10 with transient expression in COS cells.

### Example 12

#### Human $\alpha_d$ binds to ICAM-R in a CD18-dependent fashion

In view of reports that demonstrate interactions between the leukocyte integrins and intercellular adhesion molecules (ICAMs) which mediate cell-cell contact [Hynes, *Cell* 69:11-25 (1992)], the ability of CHO cells expressing  $\alpha_d$ /CD18 to bind ICAM-1, ICAM-R, or VCAM-1 was assessed by two methods.



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In replicate assays, soluble ICAM-1, ICAM-R, or VCAM-1 IgG1 fusion proteins were immobilized on plastic and the ability of  $\alpha_d$ /CD18 CHO transfected cells to bind the immobilized ligand was determined. Transfected cells were labeled internally with calcein, washed in binding buffer (RPMI with 1% BSA), and incubated in either buffer only (with or without 10 ng/ml PMA) or buffer with anti-CD18 monoclonal antibodies at 10  $\mu$ g/ml. Transfected cells were added to 96-well Immulon 4 microtiter plates previously coated with soluble ICAM-1/IgG1, ICAM-R/IgG1 or VCAM-1/IgG1 fusion protein, or bovine serum albumin (BSA) as a negative control. Design of the soluble forms of these adhesion molecules is described and fully disclosed in co-pending and co-owned U.S. Patent Application Serial No. 08/102,852, filed August 5, 1993. Wells were blocked with 1% BSA in PBS prior to addition of labeled cells. After washing the plates by immersion in PBS with 0.1% BSA for 20 minutes, total fluorescence remaining in each well was measured using a Cytofluor 2300 (Millipore, Milford, MA).

In experiments with immobilized ICAMs,  $\alpha_d$ /CD18 co-transfectants consistently showed a 3-5 fold increase in binding to ICAM-R/IgG1 wells over BSA coated wells. The specificity and CD18-dependence of this binding was demonstrated by the inhibitory effects of anti-CD18 antibody TS1/18. The binding of cells transfected with CD11a/CD18 to ICAM-1/IgG1 wells was comparable to the binding observed with BSA coated wells. CD11a/CD18 transfected cells showed a 2-3 fold increase in binding to ICAM-1/IgG1 wells only following pretreatment with PMA. PMA treatment of  $\alpha_d$ /CD18 transfectants did not affect binding to ICAM-1/IgG1 or ICAM-R/IgG1 wells. No detectable binding of  $\alpha_d$ /CD18 transfectants to VCAM-1/IgG1 wells was observed.

Binding of  $\alpha_d$ /CD18-transfected cells to soluble ICAM-1/IgG1, ICAM-R/IgG1, or VCAM-1/IgG1 fusion proteins was determined by flow cytometry. Approximately one million  $\alpha_d$ /CD18-transfected CHO cells (grown in spinner flasks for higher expression) per measurement were suspended in 100  $\mu$ l

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binding buffer (RPMI and 1% BSA) with or without 10  $\mu$ g/ml anti-CD18 antibody. After a 20 minute incubation at room temperature, the cells were washed in binding buffer and soluble ICAM-1/IgG1 or ICAM-R/IgG1 fusion protein was added to a final concentration of 5  $\mu$ g/ml. Binding was allowed to proceed for 30 minute at 37°C, after which the cells were washed three times and resuspended in 100  $\mu$ l binding buffer containing FITC-conjugated sheep anti-human IgG1 at a 1:100 dilution. After a 30 minute incubation, samples were washed three times and suspended in 200  $\mu$ l binding buffer for analysis with a Becton Dickinson FACScan.

Approximately 40-50% of the  $\alpha_d$ /CD18 transfectants indicated binding to ICAM-R/IgG1, but no binding to ICAM-1/IgG1 or VCAM-1/IgG1 proteins. Pretreatment of transfected cells with PMA has no effect on  $\alpha_d$ /CD18 binding to either ICAM-1/IgG1, ICAM-R/IgG1 or VCAM-1/IgG1, which was consistent with the immobilized adhesion assay. Binding by ICAM-R was reduced to background levels after treatment of  $\alpha_d$ /CD18 transfectants with anti-CD18 antibody TS1/18.

The collective data from these two binding assays illustrate that  $\alpha_d$ /CD18 binds to ICAM-R and does so preferentially as compared to ICAM-1 and VCAM-1. The  $\alpha_d$ /CD18 binding preference for ICAM-R over ICAM-1 is opposite that observed with CD11a/CD18 and CD11b/CD18. Thus modulation of  $\alpha_d$ /CD18 binding may be expected to selectively affect normal and pathologic immune function where ICAM-R plays a prominent role. Moreover, results of similar assays, in which antibodies immunospecific for various extracellular domains of ICAM-R were tested for their ability to inhibit binding of ICAM-R to  $\alpha_d$ /CD18 transfectants, indicated that  $\alpha_d$ /CD18 and CD11a/CD18 interact with different domains of ICAM-R.

The failure of CD11a/CD18 to bind ICAM-1/IgG1 or ICAM-R/IgG1 in solution suggests that the affinity of binding between CD11a/CD18 and ICAM-1 or ICAM-R is too low to permit binding in solution. Detection of

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$\alpha_d$ /CD18 binding to ICAM-R/IgG1, however, suggests an unusually high binding affinity.

#### $\alpha_d$ Binding to iC3b

Complement component C3 can be proteolytically cleaved to form the complex iC3b, which initiates the alternative pathway of complement activation and leads ultimately to cell-mediated destruction of a target. Both CD11b and CD11c have been implicated in iC3b binding and subsequent phagocytosis of iC3b-coated particles. A peptide fragment in the CD11b I domain has recently been identified as the site of iC3b interaction [Ueda, *et al.*, *Proc.Natl.Acad.Sci.(USA)* 91:10680-10684 (1994)]. The region of iC3b binding is highly conserved in CD11b, CD11c, and  $\alpha_d$ , suggesting an  $\alpha_d$ /iC3b binding interaction.

Binding of  $\alpha_d$  to iC3b is performed using transfectants or cell lines naturally expressing  $\alpha_d$  (for example, PMA-stimulated HL60 cells) and iC3b-coated sheep red blood cells (sRBC) in a rosette assay [Dana, *et al.*, *J.Clin.Invest.* 73:153-159 (1984)]. The abilities of  $\alpha_d$ /CD18 CHO transfectants, VLA4-CHO transfectants (negative control) and PMA-stimulated HL60 cells (positive control) to form rosettes are compared in the presence and absence of an anti-CD18 monoclonal antibody (for example TS1/18.1).

#### 20 Example 13

##### Screening by Scintillation Proximity Assay

Specific inhibitors of binding between the  $\alpha_d$  ligands of the present invention and their binding partners ( $\alpha_d$  ligand/anti-ligand pair) may be determined by a variety of means, such as scintillation proximity assay techniques as generally described in U.S. Patent No. 4,271,139, Hart and Greenwald, *Mol.Immunol.* 12:265-267 (1979), and Hart and Greenwald, *J.Nuc.Med.* 20:1062-1065 (1979), each of which is incorporated herein by reference.

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Briefly, one member of the  $\alpha_d$  ligand/anti-ligand pair is bound to a solid support. A fluorescent agent is also bound to the support. Alternatively, the fluorescent agent may be integrated into the solid support as described in U.S. Patent No. 4,568,649, incorporated herein by reference. The non-support bound member of the  $\alpha_d$  ligand/anti-ligand pair is labeled with a radioactive compound that emits radiation capable of exciting the fluorescent agent. When the ligand binds the radiolabeled anti-ligand, the label is brought sufficiently close to the support-bound fluorester to excite the fluorester and cause emission of light. When not bound, the label is generally too distant from the solid support to excite the fluorescent agent, and light emissions are low. The emitted light is measured and correlated with binding between the ligand and the anti-ligand. Addition of a binding inhibitor to the sample will decrease the fluorescent emission by keeping the radioactive label from being captured in the proximity of the solid support. Therefore, binding inhibitors may be identified by their effect on fluorescent emissions from the samples. Potential anti-ligands to  $\alpha_d$  may also be identified by similar means.

#### Example 14

##### Soluble Human $\alpha_d$ Expression Constructs

The expression of full-length, soluble human  $\alpha_d$ /CD18 heterodimeric protein provides easily purified material for immunization and binding assays. The advantage of generating soluble protein is that it can be purified from supernatants rather than from cell lysates (as with full-length membrane-bound  $\alpha_d$ /CD18); recovery is therefore improved and impurities reduced.

The soluble  $\alpha_d$  expression plasmid was constructed as follows. A nucleotide fragment corresponding to the region from bases 0 to 3161 in SEQ ID NO: 1, cloned into plasmid pATM.D12, was isolated by digestion with *HindIII* and *AarII*. A PCR fragment corresponding to bases 3130 to 3390 in SEQ ID NO:

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1, overlapping the *HindIII/AatII* fragment and containing an addition *MluI* restriction site at the 3' terminus, was amplified from pATM.D12 with primers sHAD.5 and sHAD.3 set out in SEQ ID NOS: 30 and 31, respectively.

5'-TTGCTGACTGCCTGCAGTTC-3' (SEQ ID NO: 30)

5'-GTTCTGACGCGTAATGGCATTGTAGACCTCGTCTTC (SEQ ID NO: 31)

The PCR amplification product was digested with *AatII* and *MluI* and ligated to the *HindIII/AatII* fragment. The resulting product was ligated into *HindIII/MluI*-digested plasmid pDC1.s.

This construct is co-expressed with soluble CD18 in stably transfected CHO cells, and expression is detected by autoradiographic visualization of immunoprecipitated CD18 complexes derived from <sup>35</sup>S-methionine labeled cells. The construct is also co-expressed with CD18 in 293 cells [Berman, *et al.*, *J. Cell. Biochem.* 52:183-195 (1993)].

#### Soluble Human $\alpha_d$ I Domain Expression Constructs

It has previously been reported that the I domain in CD11a can be expressed as an independent structural unit that maintains ligand binding capabilities and antibody recognition [Randi and Hogg, *J. Biol. Chem.* 269:12395-12398 (1994); Zhout, *et al.*, *J. Biol. Chem.* 269:17075-17079 (1994); Michishita, *et al.*, *Cell* 72:857-867 (1993)]. To generate a soluble fusion protein comprising the  $\alpha_d$  I domain and human IgG4, the  $\alpha_d$  I domain is amplified by PCR using primers designed to add flanking *BamHI* and *XhoI* restriction sites to facilitate subcloning. These primers are set out in SEQ ID NOS: 32 and 33 with restriction sites underlined.

5'-ACGTATGCAGGATCCCATCAAGAGATGGACATCGCT (SEQ ID NO: 32)

5'-ACTGCATGTCTCGAGGCTGAAGCCTTCTTGGGACAT (SEQ ID NO: 33)

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The C nucleotide immediately 3' to the *Bam*HI site in SEQ ID NO: 32 corresponds to nucleotide 435 in SEQ ID NO: 1; the G nucleotide 3' to the *Xho*I site in SEQ ID NO: 33 is complementary to nucleotide 1067 in SEQ ID NO: 1. The amplified I domain is digested with the appropriate enzymes, the purified  
5 fragment ligated into the mammalian expression vector pDCs and the prokaryotic expression vector pGEX-4T-3 (Pharmacia) and the I domain fragment sequenced. The fusion protein is then expressed in COS, CHO or *E. coli* cells transfected or transformed with an appropriate expression construct.

Given the affinity of  $\alpha_d$  for ICAM-R, expression of the  $\alpha_d$  I  
10 domain may be of sufficient affinity to be a useful inhibitor of cell adhesion in which  $\alpha_d$  participates.

#### Analysis of Human $\alpha_d$ I Domain/IgG4 Fusion Proteins

Protein was resolved by SDS-PAGE under reducing and non-reducing conditions and visualized by either silver staining or Coomassie staining.  
15 Protein was then transferred to Immobilon PVDF membranes and subjected to Western blot analysis using anti-human IgG monoclonal antibodies or anti-bovine Ig monoclonal antibodies.

Protein detected was determined to migrate at about 120 kD under non-reducing conditions and at about 45 kD under reducing conditions. Minor  
20 bands were also detected on non-reducing gels at approximately 40-50 kD which were reactive with the anti-human, but not anti-bovine, antibodies. A 200 kD minor band was determined to be bovine Ig by Western blot.

#### Binding Assays Using I Domain Expression Products

The ability of the I domain to specifically recognize ICAM-R/IgG  
25 chimeric protein was tested in an ELISA format. Serial dilutions of  $\alpha_d$  I domain IgG4 fusion protein ( $\alpha_d$ /IgG4) in TBS were incubated with ICAM-1/IgG, ICAM-R/IgG, VCAM-1/IgG, or an irrelevant IgG1 myeloma protein

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immobilized on Immulon IV RIA/EIA plates. CD11a I domain/IgG chimeric protein and human IgG4/kappa myeloma protein were used as negative controls. Bound IgG4 was detected with the biotinylated anti-IgG4 monoclonal antibody HP6023 followed by addition of streptavidin-peroxidase conjugate and development with substrate *o*-phenyldiamine.

In repeated assays, no binding of the CD11a/IgG4 protein or the IgG4 myeloma protein was detected with any of the immobilized proteins. The  $\alpha_d$ /IgG4 protein did not bind to fish skin gelatin or bovine serum albumin blocking agents, human IgG1, or ICAM-1/IgG. A two to three fold increase in binding signal over background was detected in ICAM-R/IgG protein coated wells using 1-5  $\mu$ g/ml concentrations of  $\alpha_d$ /IgG4 protein. The signal in VCAM-1/IgG protein coated wells was 7-10 fold higher than background. In previous assays,  $\alpha_d$ /CD18 transfected CHO cells did not bind VCAM-1/IgG protein, suggesting that VCAM-1 binding may be characteristic of isolated I domain amino acid sequences.

#### Additional $\alpha_d$ I domain constructs

Additional  $\alpha_d$  I domain constructs are generated in the same fashion as the previous construct, but incorporating more amino acids around the  $\alpha_d$  I domain. Specific constructs include: i) sequences from exon 5 (amino acids 127-353 in SEQ ID NO: 2), preceding the current construct, ii) the EF-hand repeats (amino acids 17-603 in SEQ ID NO: 2) following the I domain, and iii) the alpha chain truncated at the transmembrane region (amino acids 17-1029 in SEQ ID NO: 2), with an IgG4 tail for purification and detection purposes. These constructs are ligated into either the mammalian expression vector pDCS1 or the prokaryotic expression vector pGEX-4T-3 (Pharmacia) and the I domain sequenced. The fusion proteins are then be expressed in COS, CHO, or *E.coli* cells transformed or transfected with an appropriate expression construct. Protein are purified on a ProSepA column (Bioprocessing Limited, Durham, England),

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tested for reactivity with the anti-IgG4 monoclonal antibody HP6023 and visualized on polyacrylamide gels with Coomassie staining.

5 In order to construct an expression plasmid for the entire  $\alpha_d$  polypeptide, pATM.D12, described *supra*, is modified to express an  $\alpha_d$ -IgG4 fusion protein by the following method. IgG4 encoding DNA is isolated from the vector pDCS1 by PCR using primers which individually incorporate a 5' *Aat*II restriction site (SEQ ID NO: 89) and a 3' *Xba*I restriction site (SEQ ID NO: 90).

5'-CGCTGTGACGTCAGAGTTGAGTCCAAATATGG-3' (SEQ ID NO: 89)

5'-GGTGACACTATAGAATAGGGC-3' (SEQ ID NO: 90)

10 Plasmid pATM.D12 is digested with *Aat*II and *Xba*I, and the appropriately digested and purified IgG4 PCR product ligated into the linear vector.



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**Example 15****Production of Human  $\alpha_d$ -Specific Monoclonal Antibodies**

Transiently transfected cells from Example 7 were washed three times in Dulbecco's phosphate buffered saline (D-PBS) and injected at  $5 \times 10^6$  cells/mouse into Balb/c mice with 50  $\mu\text{g}$ /mouse muramyl dipeptidase (Sigma) in PBS. Mice were injected two more times in the same fashion at two week intervals. The pre-bleed and immunized serum from the mice were screened by FACS analysis as outlined in Example 9 and the spleen from the mouse with the highest reactivity to cells transfected with  $\alpha_d$ /CD18 was fused. Hybridoma culture supernatants were then screened separately for lack of reactivity against COS cells transfected with CD11a/CD18 and for reactivity with cells co-transfected with an  $\alpha_d$  expression plasmid and CD18.

This method resulted in no monoclonal antibodies.

As an alternative for production of monoclonal antibodies, soluble  $\alpha_d$  I domain IgG4 fusion protein was affinity purified from supernatant of stably transfected CHO cells and used to immunize Balb/c mice as described above. Hybridomas were established and supernatants from these hybridomas were screened by ELISA for reactivity against  $\alpha_d$  I domain fusion protein. Positive cultures were then analyzed for reactivity with full length  $\alpha_d$ /CD18 complexes expressed on CHO transfectants.

Mouse 1908 received three initial immunizations of  $\alpha_d$ /CD18 transfected CHO cells and two subsequent boosts with soluble  $\alpha_d$ /CD18 heterodimer. Two final immunizations included 50  $\mu\text{g}$ /mouse  $I\alpha_d$ /IgG4 fusion protein. The fusion produced 270 IgG-producing wells. Supernatant from 45 wells showed at least 7-fold higher binding to  $I\alpha_d$ /IgG4 fusion protein than to human IgG4 by ELISA. None of the supernatants reacted to  $\alpha_d$ /CD18 transfected CHO cells as determined by FACS analysis.

To determine whether the supernatants were able to recognize integrin alpha subunit proteins in another context, fresh frozen splenic sections

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were stained with supernatants from 24 of the 45 wells. Three supernatants were determined to be positive: one stained large cells in the red pulp, while two others stained scattered cells in the red pulp and also trabeculae.

5        These supernatants were further analyzed by their ability to immunoprecipitate biotinylated CD18 complexes from either  $\alpha_d$ /CD18 transfected CHO cells or PMA-stimulated HL60 cells. Fusion wells with supernatants that recognized protein in detergent lysates (which should not be as conformationally constrained as protein expressed as heterodimers) were selected for further subcloning. Monoclonal antibodies which recognize protein in detergent may be  
10        more useful in immunoprecipitation of heterodimeric complexes from transfectants, tissues, and cell lines.

      As another alternative, monoclonal antibodies are generated as follows. Affinity purified  $\alpha_d$ /CD18 heterodimeric protein from detergent lysates of stably transfected CHO cells is used with 50  $\mu$ g/ml muramyl dipeptidase to  
15        immunize Balb/c mice as described above. Mice receive three immunizations before serum reactivity against  $\alpha_d$ /CD18 is determined by immunoprecipitation of biotinylated complexes in the CHO transfectants. Hybridomas from positive animals are established according to standard protocols, after which hybridoma  
20        cultures are selected by flow cytometry using  $\alpha_d$ /CD18 transfectants. CD11a/CD18 transfectants are utilized to control for CD18-only reactivity.

      As another alternative for monoclonal antibody production, Balb/c mice undergo an immunization/immunosuppression protocol designed to reduce reactivity to CHO cell determinants on transfectants used for immunization. This  
25        protocol involves immunization with untransfected CHO cells and subsequent killing of CHO-reactive B-cell blasts with cyclophosphamide treatment. After three rounds of immunization and cyclophosphamide treatment are performed, the mice are immunized with  $\alpha_d$ /CD18 CHO transfected cells as described above.

      As still another alternative, heterodimeric CD18 complexes are immunoprecipitated from detergent lysates of whole spleen using an anti-CD18

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monoclonal antibody, following preclearance of CD11a/CD18 and CD11b/CD18. CD11a/CD18 and CD11b/CD18 complexes are precleared by affinity chromatography using monoclonal antibodies TS2/4 and Mo1, respectively, coupled to a chromatographic resin. The remaining CD18 complexes are used as an immunogen in Balb/c mice for the first immunization. Three immunizations are given at three week intervals, the initial immunization administered in conjunction with Freund's Complete Adjuvant and the subsequent immunizations with Freund's Incomplete Adjuvant. Serum is assayed for  $\alpha_d$ -specific reactivity by immunoprecipitation. Resulting hybridomas are screened by flow cytometry with  $\alpha_d$ /CD18 CHO transfectants.

As another alternative, CD18 complexes from detergent lysates of PMA stimulated HL60 cells are enriched by preclearance as described above. Other  $\beta 2$  integrins are cleared on the same columns. Immunization with the resulting complexes, hybridoma production, and screening protocols are performed as described *supra*.

#### Example 16

##### Analysis of $\alpha_d$ distribution with polyclonal serum

Tissue distribution of  $\alpha_d$ /CD18 was determined using polyclonal antiserum. Antiserum used to stain tissue was obtained from a mouse immunized 3 times with  $\alpha_d$  transfectected CHO cells (D6.CHO,  $\alpha_d$ /CD18) with adjuvant peptide and once with purified  $\alpha_d$ /CD18 heterodimer. A final boost included only  $\alpha_d$ /CD18 heterodimer. Approximately 100  $\mu$ l immunized serum was precleared by addition of approximately  $10^8$  LFA-1-transfected CHO cells for 2 hours at 4°C. The resulting serum was assayed for  $\alpha_d$  reactivity at dilutions of 1/5000, 1/10000, 1/20000 and 1/40000 on normal human spleen. The polyclonal antibody was reactive at a dilution of 1/20000, while a 1/40000 dilution stained very weakly.

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Once serum was determined to have specific  $\alpha_d$  reactivity, it was used to stain various lymphoid and non-lymphoid tissues. Monoclonal antibodies recognizing CD18, CD11a, CD11b, and CD11c were used in the same experiment as controls. Staining of normal spleen sections with  $\alpha_d$  polyclonal sera, and monoclonal antibodies to CD11a, CD11b, CD11c, and CD18 revealed the following results. The pattern observed with  $\alpha_d$  polyclonal sera did not display the same pattern of labeling as CD11a, CD11b, CD11c, or CD18. There is a distinct pattern of labeling with some cells located in the marginal zone of the white pulp and a distinct labeling of cells peripheral to the marginal zone. This pattern was not observed with the other antibodies. Individual cells scattered throughout the red pulp were also labeled which may or may not be the same population or subset seen with CD11a and CD18.

Labeling with CD11c did display some cells staining in the marginal zone, but the antibody did not show the distinct ring pattern around the white pulp when compared to  $\alpha_d$  polyclonal sera, nor did labeling in the red pulp give the same pattern of staining as  $\alpha_d$  polyclonal sera.

Therefore, the labeling pattern seen with  $\alpha_d$  polyclonal serum was unique compared to that seen using antibodies to the other  $\beta_2$  integrins (CD11a, CD11b, CD11c, and CD18), and suggests that the *in vivo* distribution of  $\alpha_d$  in man is distinct from that of other  $\beta_2$  integrins.

#### Example 17

##### Isolation of Rat cDNA Clones

In view of the existence of both canine and human  $\alpha_d$  subunits, attempts were made to isolate homologous genes in other species, including rat (this example) and mouse (Example 17, *infra*).

A partial sequence of a rat cDNA showing homology to the human  $\alpha_d$  gene was obtained from a rat splenic  $\lambda$ gt10 library (Clontech). The library was plated at  $2 \times 10^4$  pfu/plate onto 150 mm LBM/agar plates. The library was

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lifted onto Hybond membranes (Amersham), denatured 3 minutes, neutralized 3 minutes and washed 5 minutes with buffers as described in standard protocols [Sambrook, *et al.*, Molecular Cloning: a laboratory manual, p.2.110]. The membranes were placed immediately into a Stratalinker (Stratagene) and the DNA crosslinked using the autocrosslinking setting. The membranes were prehybridized and hybridized in 30% or 50% formamide, for low and high stringency conditions, respectively. Membranes were initially screened with a <sup>32</sup>P-labeled probe generated from the human  $\alpha_d$  cDNA, corresponding to bases 500 to 2100 in clone 19A2 (SEQ ID NO: 1). The probe was labeled using Boehringer Mannheim's Random Prime Kit according to manufacturer's suggested protocol. Filters were washed with 2X SSC at 55°C.

Two clones, designated 684.3 and 705.1, were identified which showed sequence homology to human  $\alpha_d$ , human CD11b, and human CD11c. Both clones aligned to the human  $\alpha_d$  gene in the 3' region of the gene, starting at base 1871 and extending to base 3012 for clone 684.3, and bases 1551 to 3367 for clone 705.1.

In order to isolate a more complete rat sequence which included the 5' region, the same library was rescreened using the same protocol as employed for the initial screening, but using a mouse probe generated from clone A1160 (See Example 17, *infra*). Single, isolated plaques were selected from the second screening and maintained as single clones on LBM/agar plates. Sequencing primers 434FL and 434FR (SEQ ID NOS: 34 and 35, respectively) were used in a standard PCR protocol to generate DNA for sequencing.

5'-TATAGACTGCTGGGTAGTCCCCAC-3' (SEQ ID NO: 34)

25 5'-TGAAGATTGGGGGTAAATAACAGA-3' (SEQ ID NO: 35)

DNA from the PCR was purified using a Quick Spin Column (Qiagen) according to manufacturer's suggested protocol.

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Two clones, designated 741.4 and 741.11, were identified which overlapped clones 684.3 and 705.1; in the overlapping regions, clones 741.1 and 741.11 were 100% homologous to clones 684.3 and 705.1. A composite rat cDNA having homology to the human  $\alpha_d$  gene is set out in SEQ ID NO: 36; the predicted amino acid sequence is set forth in SEQ ID NO: 37.

Cloning of the 5' end of Rat  $\alpha_d$

A 5' cDNA fragment for the rat  $\alpha_d$  gene was obtained using a Clontech rat spleen RACE cloning kit according to manufacturer's suggested protocol. The gene specific oligonucleotides used were designated 741.11#2R and 741.2#1R (SEQ ID NOS: 59 and 58, respectively).

5'-CCAAAGCTGGCTGCATCCTCTC-3' (SEQ ID NO: 59)

5'-GGCCTTGCAGCTGGACAATG-3' (SEQ ID NO: 58)

Oligo 741.11#2R encompasses base pairs 131-152 in SEQ ID NO: 36, in the reverse orientation and 741.2#1R encompasses bases pairs 696-715 in SEQ ID NO: 36, also in the reverse orientation. A primary PCR was carried out using the 3'-most oligo, 741.2#1R. A second PCR followed using oligo 741.11#2R and DNA generated from the primary reaction. A band of approximately 300 base pairs was detected on a 1% agarose gel.

The secondary PCR product was ligated into plasmid pCRTAI (Invitrogen) according to manufacturer's suggested protocol. White (positive) colonies were picked and added to 100  $\mu$ l LBM containing 1  $\mu$ l of a 50 mg/ml carbenicillin stock solution and 1  $\mu$ l M13 K07 phage culture in individual wells in a round bottom 96 well tissue culture plate. The mixture was incubated at 37°C for 30 minutes to one hour. Following the initial incubation period, 100  $\mu$ l of LBM (containing 1  $\mu$ l of 50 mg/ml carbenicillin and a 1:250 dilution of a 10

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mg/ml kanamycin stock solution) were added and the incubation was continued overnight at 37°C.

Using a sterile 96 well metal transfer prong, supernatant from the 96 well plate was transferred to four Amersham Hybond nylon filters. The filters were denatured, neutralized and cross linked by standard protocols. The filters were prehybridized in 20 mls of prehybridization buffer (5X SSPE; 5X Denhardt's; 1% SDS; 50 ugs/ml denatured salmon sperm DNA) at 50°C for several hours while shaking.

Oligo probes 741.11#1 and 741.11#1R (SEQ ID NOS: 56 and 57, respectively), encompassing base pairs 86-105 (SEQ ID NO: 36) in the forward and reverse orientation respectively, were labeled as follows.

5'-CCTGTCATGGGTCTAACCTG-3' (SEQ ID NO: 56)

5'-AGGTTAGACCCATGACAGG-3' (SEQ ID NO: 57)

Approximately 65 ng oligo DNA in 12  $\mu$ l dH<sub>2</sub>O was heated to 65°C for two minutes. Three  $\mu$ l of 10 mCi/ml  $\gamma$ -<sup>32</sup>P-ATP were added to the tube along with 4  $\mu$ l 5x Kinase Buffer (Gibco) and 1  $\mu$ l T4 DNA Kinase (Gibco). The mixture was incubated at 37°C for 30 minutes. Following incubation, 16  $\mu$ l of each labeled oligo probe were added to the prehybridization buffer and filters and hybridization was continued overnight at 42°C. The filters were washed three times in 5X SSPE; 0.1% SDS for 5 minutes per wash at room temperature, and autoradiographed for 6 hours. Positive clones were expanded and DNA purified using the Magic Mini Prep Kit (Promega) according to manufacturer's suggested protocol. Clone 2F7 was selected for sequencing and showed 100% homology to clone 741.11 in the overlapping region. The complete rat  $\alpha_d$  nucleic acid sequence is set out in SEQ ID NO: 54; the amino acid sequence is set out in SEQ ID NO: 55.

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### Characteristics of the Rat cDNA and Amino Acid Sequences

Neither nucleic acid nor amino acid sequences have previously been reported for rat  $\alpha$  subunits in  $\beta_2$  integrins. However sequence comparisons to reported human  $\beta_2$  integrin  $\alpha$  subunits suggests that the isolated rat clone and its predicted amino acid sequence are most closely related to  $\alpha_d$  nucleotide and amino acid sequences.

At the nucleic acid level, the isolated rat cDNA clone shows 80% identity in comparison to the human  $\alpha_d$  cDNA; 68% identity in comparison to human CD11b; 70% identity in comparison to human CD11c; and 65% identity in comparison to mouse CD11b. No significant identity is found in comparison to human CD11a and to mouse CD11a.

At the amino acid level, the predicted rat polypeptide encoded by the isolated cDNA shows 70% identity in comparison to human  $\alpha_d$  polypeptide; 28% identity in comparison to human CD11a; 58% identity in comparison to human CD11b; 61% identity in comparison to human CD11c; 28% identity in comparison to mouse CD11a; and 55% identity in comparison to mouse CD11b.

### Example 18

#### Monoclonal Antibodies against Rat $\alpha_d$ I domain/Hu IgG4 Fusion Proteins

In view of the fact that the I domain of human  $\beta_2$  integrins has been demonstrated to participate in ligand binding, it was assumed that the same would be true for rat  $\alpha_d$  protein. Monoclonal antibodies immunospecific for the rat  $\alpha_d$  I domain may therefore be useful in rat models of human disease states wherein  $\alpha_d$  binding is implicated.

Oligos "rat alpha-DI5" (SEQ ID NO: 87) and "rat alpha-DI3" (SEQ ID NO: 88) were generated from the rat  $\alpha_d$  sequence corresponding to base pairs 469-493 and base pairs 1101-1125 (in the reverse orientation), respectively, in SEQ ID NO: 54. The oligos were used in a standard PCR reaction to generate a rat  $\alpha_d$  DNA fragment containing the I domain spanning base pairs 459-1125 in



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SEQ ID NO: 54. The PCR product was ligated into vector pCRTAII (Invitrogen) according to manufacturer's suggested protocol. A positive colony was selected and expanded for DNA purification using a Qiagen (Chatsworth, GA) Midi Prep kit according to manufacturer's protocol. The DNA was digested with *XhoI* and *BglIII* in a standard restriction enzyme digest and a 600 base pair band was gel purified which was subsequently ligated into pDCS1/HuIgG4 expression vector. A positive colony was selected, expanded and DNA purified with a Qiagen Maxi Prep Kit.

COS cells were plated at half confluence on 100mm culture dishes and grown overnight at 37°C in 7% CO<sub>2</sub>. Cells were rinsed once with 5 ml DMEM. To 5 ml DMEM, 50 µl DEAE-Dextran, 2 µl chloroquine and 15 µg rat α<sub>4</sub> I domain/HuIgG4 DNA described above was added. The mixture was added to the COS cells and incubated at 37°C for 3 hours. Media was then removed and 5 ml 10% DMSO in CMF-PBS was added for exactly one minute. The cells were gently rinsed once with DMEM. Ten ml DMEM containing 10% FBS was added to the cells and incubation continued overnight at 37°C in 7% CO<sub>2</sub>. The next day, media was replaced with fresh media and incubation continued for three additional days. The media was harvested and fresh media was added to the plate. After three days, the media was collected again and the plates discarded. The procedure was repeated until 2 liters of culture supernatant were collected.

Supernatant collected as described above was loaded onto a Prosep-A column (Bioprocessing Limited) and protein purified as described below.

The column was initially washed with 15 column volumes of Wash Buffer containing 35 mM Tris and 150 mM NaCl, pH 7.5. Supernatant was loaded at a slow rate of less than approximately 60 column volumes per hour. After loading, the column was washed with 15 column volumes of Wash Buffer, 15 column volumes of 0.55 M diethanolamine, pH 8.5, and 15 column volumes

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50 mM citric acid, pH 5.0. Protein was eluted with 50 mM citric acid, pH 3.0. Protein was neutralized with 1.0 M Tris, pH 8.0, and dialyzed in sterile PBS.

The rat  $\alpha_d$  I domain protein was analyzed as described in Example 14. The detected protein migrated in the same manner as observed with human I domain protein.

#### Immunization Protocol

Mice were individually immunized with 50  $\mu$ g purified rat  $\alpha_d$  I domain/HuIgG4 fusion protein previously emulsified in an equal volume of Freund's Complete Adjuvant (FCA) (Sigma). Approximately 200  $\mu$ l of the antigen/adjuvant preparation was injected at 4 sites in the back and flanks of each of the mice. Two weeks later the mice were boosted with an injection of 100  $\mu$ l rat  $\alpha_d$  I domain/HuIgG4 antigen (50  $\mu$ g/mouse) previously emulsified in an equal volume of Freund's Incomplete Adjuvant (FIA). After two additional weeks, the mice were boosted with 50  $\mu$ g antigen in 200  $\mu$ l PBS injected intravenously.

To evaluate serum titers in the immunized mice, retro-orbital bleeds were performed on the animals ten days following the third immunization. The blood was allowed to clot and serum isolated by centrifugation. The serum was used in an immunoprecipitation on biotinylated (BIP) rat splenocytes. Serum from each mouse immunoprecipitated protein bands of expected molecular weight for rat  $\alpha_d$  and rat CD18. One mouse was selected for the fusion and was boosted a fourth time as described above for the third boost.

The hybridoma supernatants were screened by antibody capture, described as follows. Immulon 4 plates (Dynatech, Cambridge, Massachusetts) were coated at 4°C with 50  $\mu$ l/well goat anti-mouse IgA, IgG or IgM (Organon Teknika) diluted 1:5000 in 50 mM carbonate buffer, pH 9.6. Plates were washed 3X with PBS containing 0.05% Tween 20 (PBST) and 50  $\mu$ l culture supernatant was added. After incubation at 37°C for 30 minutes, and washing as described above, 50  $\mu$ l horseradish peroxidase-conjugated goat anti-mouse IgG9(Fc)

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(Jackson ImmunoResearch, West Grove, Pennsylvania) diluted 1:3500 in PBST was added. Plates were incubated as described above and washed 4X with PBST. Immediately thereafter, 100  $\mu$ l substrate, containing 1 mg/ml *o*-phenylene diamine (Sigma) and 0.1  $\mu$ l/ml 30% H<sub>2</sub>O<sub>2</sub> in 100 mM citrate, pH4.5, was added. The color reaction was stopped after 5 minutes with the addition of 50  $\mu$ l 15% H<sub>2</sub>SO<sub>4</sub>. Absorbance at 490 nm was read on a Dynatech plate reader.

Supernatant from antibody-containing wells was also analyzed by ELISA with immobilized rat  $\alpha$ <sub>d</sub> I domain/HuIgG4 fusion protein. An ELISA with HuIgG4 antibody coated plates served as a control for reactivity against the IgG fusion partner. Positive wells were selected for further screening by BIP on rat splenocyte lysates using techniques described below.

#### Biotinylation of Cell Surface Antigens

Rats were sacrificed by asphyxiation with CO<sub>2</sub> and spleens were removed using standard surgical techniques. Splenocytes were harvested by gently pushing the spleen through a wire mesh with a 3 cc syringe plunger in 20 ml RPMI. Cells were collected into a 50 ml conical tube and washed in the appropriate buffer.

Cells were washed three times in cold D-PBS and resuspended at a density of 10<sup>8</sup> to 10<sup>9</sup> cells in 40 ml PBS. Four mg of NHS-Biotin (Pierce) was added to the cell suspension and the reaction was allowed to continue for exactly 15 minutes at room temperature. The cells were pelleted and washed three times in cold D-PBS.

#### Cell Lysates

Cells were resuspended at a density of 10<sup>8</sup> cells/ml in cold lysis Buffer (1% NP40; 50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 2 mM CaCl<sub>2</sub>; 2 mM MgCl<sub>2</sub>; 1:100 solution of pepstatin, leupeptine, and aprotinin, added just before adding to cells; and 0.0001 g PMSF crystals, added just before adding to cells).

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Lysates were vortexed for approximately 30 seconds, incubated for 5 minute at room temperature, and further incubated for 15 minutes on ice. Lysates were centrifuged for 10 minutes at 10,000 xg to pellet the insoluble material. Supernatant was collected into a new tube and stored at between 4°C and -20°C.

5     Immunoprecipitation

One ml cell lysate was precleared by incubation with 200  $\mu$ l of a protein A sepharose slurry (Zymed) overnight at 4°C. Precleared lysate was aliquoted into Eppendorf tubes at 50  $\mu$ l/tube for each antibody to be tested. Twenty-five  $\mu$ l of polyclonal serum or 100 to 500  $\mu$ l of monoclonal antibody  
10     supernatant were added to the precleared lysates and the resulting mixture incubated for 2 hours at 4°C with rotation. One hundred  $\mu$ l rabbit anti-mouse IgG (Jackson) bound to protein A sepharose beads in a PBS slurry was then added and incubation continued for 30 minutes at room temperature with rotation. Beads were pelleted with gentle centrifugation, and washed three times with cold  
15     Wash Buffer (10 mM HEPES; 0.2 M NaCl; 1% Triton X-100). Supernatant was removed by aspiration, and 20  $\mu$ l 2X SDS sample buffer containing 10%  $\beta$ -mercaptoethanol was added. The sample was boiled for 2 minutes in a water bath, and the sample loaded onto a 5% SDS PAGE gel. Following separation, the proteins were transferred to nitrocellulose at constant current overnight. The  
20     nitrocellulose filters were blocked with 3% BSA in TBS-T for 1 hour at room temperature and the blocking buffer was removed. A 1:6000 dilution of Streptavidin-HRP conjugate (Jackson) in 0.1% BSA TBS-T was added and incubation continued for 30 minutes at room temperature. Filters were washed three times for 15 minutes each with TBS-T and autoradiographed using  
25     Amersham's ECL kit according to manufacturer's suggested protocol.

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Example 19Isolation of Mouse cDNA Clones

Isolation of a mouse  $\alpha_d$  homolog was attempted.

Cross-species hybridization was performed using two PCR-generated probes: a 1.5 kb fragment corresponding to bases 522 to 2047 from human clone 19A2 (SEQ ID NO: 1), and a 1.0 kb rat fragment which corresponds to bases 1900 to 2900 in human clone 19A2 (SEQ ID NO: 1). The human probe was generated by PCR using primer pairs designated ATM-2 and 9-10.1 set out in SEQ ID NOS: 38 and 39, respectively; the rat probe was generated using primer pairs 434L and 434R, set out in SEQ ID NOS: 34 and 35, respectively. Samples were incubated at 94°C for 4 minutes and subjected to 30 cycles of the temperature step sequence: 94°C; 50°C 2 minutes; 72°C, 4 minutes.

5'-GTCCAAGCTGTCATGGGCCAG-3' (SEQ ID NO: 38)

5'-GTCCAGCAGACTGAAGAGCACGG-3' (SEQ ID NO: 39)

The PCR products were purified using the Qiagen Quick Spin kit according to manufacturer's suggested protocol, and approximately 180 ng DNA was labeled with 200  $\mu$ Ci [ $^{32}$ P]-dCTP using a Boehringer Mannheim Random Primer Labeling kit according to manufacturer's suggested protocol. Unincorporated isotope was removed using a Centri-sep Spin Column (Princeton Separations, Adelphia, NJ) according to manufacturer's suggested protocol. The probes were denatured with 0.2 N NaOH and neutralized with 0.4 M Tris-HCl, pH 8.0, before use.

A mouse thymic oligo dT-primed cDNA library in lambda ZAP II (Stratagene) was plated at approximately 30,000 plaques per 15 cm plate. Plaque lifts on nitrocellulose filters (Schleicher & Schuell, Keene, NH) were incubated at 50°C with agitation for 1 hour in a prehybridization solution (8 ml/lift) containing 30% formamide. Labeled human and rat probes were added to the

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prehybridization solution and incubation continued overnight at 50°C. Filters were washed twice in 2X SSC/0.1% at room temperature, once in 2X SSC/0.1% SDS at 37°C, and once in 2X SSC/0.1% SDS at 42°C. Filters were exposed on Kodak X-Omat AR film at -80°C for 27 hours with an intensifying screen.

5 Four plaques giving positive signals on duplicate lifts were restreaked on LB medium with magnesium (LBM)/carbenicillin (100 mg/ml) plates and incubated overnight at 37°C. The phage plaques were lifted with Hybond filters (Amersham), probed as in the initial screen, and exposed on Kodak X-Omat AR film for 24 hours at -80°C with an intensifying screen.

10 Twelve plaques giving positive signals were transferred into low Mg<sup>++</sup> phage diluent containing 10 mM Tris-HCl and 1 mM MgCl<sub>2</sub>. Insert size was determined by PCR amplification using T3 and T7 primers (SEQ ID NOS: 13 and 14, respectively) and the following reaction conditions. Samples were incubated at 94°C for 4 minutes and subjected to 30 cycles of the temperature  
15 step sequence: 94°C, for 15 seconds; 50°C, for 30 seconds; and 72°C for 1 minute.

Six samples produced distinct bands that ranged in size from 300 bases to 1 kb. Phagemids were released via co-infection with helper phage and recircularized to generate Bluescript SK<sup>-</sup> (Stratagene). The resulting colonies  
20 were cultured in LBM/carbenicillin (100 mg/ml) overnight. DNA was isolated with a Promega Wizard miniprep kit (Madison, WI) according to manufacturer's suggested protocol. *Eco*RI restriction analysis of purified DNA confirmed the molecular weights which were detected using PCR. Insert DNA was sequenced with M13<sup>+</sup> and M13 reverse.1 primers set out in SEQ ID NOS: 40 and 41,  
25 respectively.

5'-TGTAACACGACGGCCAGT-3' (SEQ ID NO: 40)

5'-GGAAACAGCTATGACCATG-3' (SEQ ID NO: 41)

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Sequencing was performed as described in Example 4.

Of the six clones, only two, designated 10.3-1 and 10.5-2, provided sequence information and were identical 600 bp fragments. The 600 bp sequence was 68% identical to a corresponding region of human  $\alpha_d$ , 40% identical to human CD11a, 58% identical to human CD11c, and 54% identical to mouse CD11b. This 600 bp fragment was then utilized to isolate a more complete cDNA encoding a putative mouse  $\alpha_d$  homolog.

A mouse splenic cDNA library (oligo dT<sup>-</sup> and random-primed) in lambda Zap II (Stratagene) was plated at  $2.5 \times 10^4$  phage/15 cm LBM plate. Plaques were lifted on Hybond nylon transfer membranes (Amersham), denatured with 0.5 M NaOH/1.5 M NaCl, neutralized with 0.5 M Tris Base/1.5 M NaCl/11.6 HCl, and washed in 2X SSC. The DNA was cross-linked to filters by ultraviolet irradiation.

Approximately 500,000 plaques were screened using probes 10.3-1 and 10.5-2 previously labeled as described *supra*. Probes were added to a prehybridization solution and incubated overnight at 50°C. The filters were washed twice in 2X SSC/0.1% SDS at room temperature, once in 2X SSC/0.1% SDS at 37°C, and once in 2X SSC/0.1% SDS at 42°C. Filters were exposed on Kodak X-Omat AR film for 24 hours at -80°C with an intensifying screen. Fourteen plaques giving positive signals on duplicate lifts were subjected to a secondary screen identical to that for the initial screen except for additional final high stringency washes in 2X SSC/ 0.1% SDS at 50°C, in 0.5X SSC/0.1% SDS at 50°C, and at 55°C in 0.2X SSC/0.1% SDS. The filters were exposed on Kodak X-Omat AR film at -80°C for 13 hours with an intensifying screen.

Eighteen positive plaques were transferred into low Mg<sup>++</sup> phage diluent and insert size determined by PCR amplification as described above. Seven of the samples gave single bands that ranged in size from 600 bp to 4 kb. *EcoRI* restriction analysis of purified DNA confirmed the sizes observed from

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PCR and the DNA was sequenced with primers M13 and M13 reverse.1 (SEQ ID NOS: 40 and 41, respectively).

One clone designated B3800 contained a 4 kb insert which corresponded to a region 200 bases downstream of the 5' end of the human  $\alpha_d$  19A2 clone and includes 553 bases of a 3' untranslated region. Clone B3800 showed 77% identity to a corresponding region of human  $\alpha_d$ , 44% identity to a corresponding region of human CD11a, 59% identity to a corresponding region of human CD11c, and 51% identity to a corresponding region of mouse CD11b. The second clone A1160 was a 1.2 kb insert which aligned to the 5' end of the coding region of human  $\alpha_d$  approximately 12 nucleic acids downstream of the initiating methionine. Clone A1160 showed 75% identity to a corresponding region of human  $\alpha_d$ , 46% identity to a corresponding region of human CD11a, 62% identity to a corresponding region of human CD11c, and 66% identity to a corresponding region of mouse CD11b.

Clone A1160, the fragment closer to the 5' end of human clone 19A2, is 1160 bases in length, and shares a region of overlap with clone B3800 starting at base 205 and continuing to base 1134. Clone A1160 has a 110-base insertion (bases 704-814 of clone A1160) not present in the overlapping region of clone B3800. This insertion occurs at a probable exon-intron boundary [Fleming, *et al.*, *J.Immunol.* 150:480-490 (1993)] and was removed before subsequent ligation of clones A1160 and B3800.

#### Rapid Amplification of 5' cDNA End of the Putative Mouse $\alpha_d$ Clone

RACE PCR [Frohman, "RACE: Rapid Amplification of cDNA Ends," in PCR Protocols: A Guide to Methods and Applications, Innis, *et al.* (eds.) pp. 28-38, Academic Press: New York (1990)] was used to obtain missing 5' sequences of the putative mouse  $\alpha_d$  clone, including 5' untranslated sequence and initiating methionine. A mouse splenic RACE-Ready kit (Clontech, Palo Alto, CA) was used according to the manufacturer's suggested protocol. Two



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antisense, gene-specific primers, A1160 RACE1-primary and A1160 RACE2-nested (SEQ ID NOS: 42 and 43), were designed to perform primary and nested PCR.

5'-GGACATGTTCACTGCCTCTAGG-3' (SEQ ID NO: 42)

5 5'-GGCGGACAGTCAGACGACTGTCCTG-3' (SEQ ID NO: 43)

The primers, SEQ ID NOS: 42 and 43, correspond to regions starting 302 and 247 bases from the 5' end, respectively. PCR was performed as described, *supra*, using the 5' anchor primer (SEQ ID NO: 44) and mouse spleen cDNA supplied with the kit.

10 5'-CTGGTTCGGCCACCTCTGAAGGTTCCAGAATCGAT-3' (SEQ ID NO: 44)

Electrophoresis of the PCR product revealed a band approximately 280 bases in size, which was subcloned using a TA cloning kit (Invitrogen) according to manufacturer's suggested protocol. Ten resulting colonies were cultured, and the DNA isolated and sequenced. An additional 60 bases of 5' sequence were identified by this method, which correspond to bases 1 to 60 in SEQ ID NO: 45.

#### Characteristics of the Mouse cDNA and Predicted Amino Acid Sequence

A composite sequence of the mouse cDNA encoding a putative homolog of human  $\alpha_4$  is set out in SEQ ID NO: 45. Although homology between the external domains of the human and mouse clones is high, homology between the cytoplasmic domains is only 30%. The observed variation may indicate C-terminal functional differences between the human and mouse proteins. Alternatively, the variation in the cytoplasmic domains may result from splice variation, or may indicate the existence of an additional  $\beta_2$  integrin gene(s).

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At the amino acid level, the mouse cDNA predicts a protein (SEQ ID NO: 46) with 28% identity to mouse CD11a, 53% identity to mouse CD11b, 28% identity to human CD11a, 55% identity to human CD11b, 59% identity to human CD11c, and 70% identity to human  $\alpha_d$ . Comparison of the amino acid sequences of the cytoplasmic domains of human  $\alpha_d$  and the putative mouse homolog indicates regions of the same length, but having divergent primary structure. Similar sequence length in these regions suggests species variation rather than splice variant forms. When compared to the predicted rat polypeptide, Example 16, *supra*, mouse and rat cytoplasmic domains show greater than 60% identity.

#### Example 20

##### Isolation of additional mouse $\alpha_d$ cDNA clones for sequence verification

In order to verify the nucleic and amino acids sequences describe in Example 19 for mouse  $\alpha_d$ , additional mouse sequences were isolated for the purposes of confirmation.

Isolation of mouse cDNA by hybridization with two homologous  $\alpha_d$  probes (3' and 5') was performed using both a mouse splenic random primed library and an oligo dT-primed cDNA library in lambda ZAP II (Stratagene). The library was plated at  $5 \times 10^5$  phage per 15 cm LBM plate. Plaques were lifted on Hybond nylon membranes (Amersham), and the membranes were denatured (0.5 M NaOH/1.5 M NaCl), neutralized (0.5 M Tris Base/1.5 M NaCl / 11.6 M HCl) and washed (2X SSC salt solution). DNA was cross-linked to filters by ultraviolet irradiation.

Probes were generated using primers described below in a PCR reaction under the following conditions. Samples were held at 94°C for 4 minutes and then run through 30 cycles of the temperature step sequence (94°C for 15 seconds; 50°C for 30 seconds; 72°C for 1 minute in a Perkin-Elmer 9600 thermocycler).

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The 3' probe was approximately 900 bases long and spanned a region from nucleotides 2752 to 3651 (in SEQ ID NO: 1) (5' → 3') and was produced with primers 11.b-1/2FOR11 and 11.b-1/2REV2 as shown in SEQ ID NOS: 69 and 74, respectively. This probe was used in a first set of lifts.

5       The 5' probe was approximately 800 bases long and spanned a region from nucleotides 149 to 946 (in SEQ ID NO: 1) (5' → 3') and was produced with primers 11.b-1/2FOR1 and 11.a-1/1REV1 as shown in SEQ ID NOS: 50 and 85, respectively). This probe was used in a second set of lifts.

10       In a third set of lifts, both probes described above were used together on the same plates.

15       Approximately 500,000 plaques were screened using the two probes from above which were labeled in the same way as described in Example 17. Labeled probes were added to a prehybridization solution, containing 45% formamide, and incubated overnight at 50°C. Filters were washed twice in 2X SSC/0.1% SDS at room temperature (22°C). A final wash was carried out in 2X SSC/0.1% SDS at 50°C. Autoradiography was for 19 hours at -80°C on Kodak X-Omat AR film with an intensifying screen.

20       Thirteen plaques giving positive signals on at least duplicate lifts were subjected to a secondary screen performed as described for the initial screen except that both the 3' and 5' labeled probes were used for hybridization and an additional final wash was incorporated using 2X SSC/0.1% SDS at 65°C. Autoradiography was performed as described above for 2.5 hours.

25       Thirteen plaques (designated MS2P1 through MS2P13) giving positive signals were transferred into low Mg<sup>++</sup> phage diluent. Insert size was determined by PCR amplification (Perkin-Elmer 9600 thermocycler) using T3 and T7 primers which anneal to Bluescript phagemid in ZAP II (sequence previously described) under the same conditions shown above. Band sizes ranged from 500 bases to 4Kb. Phagemids were isolated, prepared, and sequenced with M13 and M13 reverse.1 primers (SEQ ID NOS: 40 and 41, respectively). Five of the

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thirteen clones; MS2P-3, MS2P-6, MS2P-9, MS2P-12, and MS2P-13, were sequenced, and together, represented a region from approximately base 200 at the 5' end to about 300 bases past a first stop codon at the 3' end.

Automated sequencing was performed as described in Example 4  
 5 by first using M13 and M13 reverse.1 primers (SEQ ID NOS: 40 and 41, respectively) to sequence the ends of each clone and to determine its position relative to construct #17 (SEQ ID NO: 45). Each clone was then completely sequenced using the appropriate primers (listed below) for that particular region.

	11.b-1/2FOR1	5'-GCAGCCAGCTTCGGACAGAC-3'	(SEQ ID NO: 50)
10	11.a-1/1FOR2	5'-CCGCCTGCCACTGGCGTGTGC-3'	(SEQ ID NO: 60)
	11.a-1/1FOR3	5'-CCCAGATGAAGGACTTCGTCAA-3'	(SEQ ID NO: 61)
	11.b-1/2FOR4	5'-GCTGGGATCATTCGCTATGC-3'	(SEQ ID NO: 62)
	11.b-1/2FOR5	5'-CAATGGATGGACCAGTTCTGG-3'	(SEQ ID NO: 63)
	11.b-1/2FOR6	5'-CAGATCGGCTCCTACTTTGG-3'	(SEQ ID NO: 64)
15	11.b-1/2FOR7	5'-CATGGAGCCTCGAGACAGG-3'	(SEQ ID NO: 65)
	11.b-1/2FOR8	5'-CCACTGTCCTCGAAGCTGGAG-3'	(SEQ ID NO: 66)
	11.b-1/2FOR9	5'-CTTCGTCCTGTGCTGGCTGTGGGCTC-3'	(SEQ ID NO: 67)
	11.b-1/2FOR10	5'-CGCCTGGCATGTGAGGCTGAG-3'	(SEQ ID NO: 68)
20	11.b-1/2FOR11	5'-CCGTGATCAGTAGGCAGGAAG-3'	(SEQ ID NO: 69)
	11.b-1/2FOR12	5'-GTCACAGAGGGAACCTCC-3'	(SEQ ID NO: 70)
	11.b-1/2FOR13	5'-GCTCCTGAGTGAGGCTGAAATCA-3'	(SEQ ID NO: 71)
	11.b-1/2FOR14	5'-GAGATGCTGGATCTACCATCTGC-3'	(SEQ ID NO: 72)
	11.b-1/2FOR15	5'-CTGAGCTGGGAGATTTTTATGG-3'	(SEQ ID NO: 73)
25	11.b-1/2REV2	5'-GTGGATCAGCACTGAAATCTG-3'	(SEQ ID NO: 74)
	11.b-1/2REV3	5'-CGTTTGAAGAAGCCAAGCTTG-3'	(SEQ ID NO: 75)
	11.b-1/2REV4	5'-CACAGCGGAGGTGCAGGCAG-3'	(SEQ ID NO: 76)
	11.b-1/2REV5	5'-CTCACTGCTTGCGCTGGC-3'	(SEQ ID NO: 77)

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11.b-1/2REV6 5'-CGGTAAGATAGCTCTGCTGG-3' (SEQ ID NO: 78)  
 11.b-1/2REV7 5'-GAGCCCACAGCCAGCACAGG-3' (SEQ ID NO: 79)  
 11.b-1/2REV8 5'-GATCCAACGCCAGATCATACC-3' (SEQ ID NO: 80)  
 11.b-1/2REV9 5'-CACGGCCAGGTCCACCAGGC-3' (SEQ ID NO: 81)  
 5 11.b-1/2REV10 5'-CACGTCCCCTAGCACTGTCAG-3' (SEQ ID NO: 82)  
 11.b-1/2REV11 5'-CCATGTCCACAGAACAGAGAG-3' (SEQ ID NO: 51)  
 11.b-1/2REV12 5'-TTGACGAAGTCCTTCATCTGGG-3' (SEQ ID NO: 83)  
 11.b-1/2REV13 5'-GAACTGCAAGCTGGAGCCCAG-3' (SEQ ID NO: 84)  
 11.a-1/1REV1 5'-CTGGATGCTGCGAAGTGCTAC-3' (SEQ ID NO: 85)  
 10 11.a-1/1REV2 5'-GCCTTGGAGCTGGACGATGGC-3' (SEQ ID NO: 86)

Sequences were edited, aligned, and compared to a previously isolated mouse  $\alpha_d$  sequence (construct #17, SEQ ID NO: 45).

Alignment of the new sequences revealed an 18 base deletion in  
 construct #17 beginning at nucleotide 2308; the deletion did not cause a shift in  
 15 the reading frame. Clone MS2P-9, sequenced as described above, also revealed  
 the same 18 base deletion. The deletion has been observed to occur in 50% of  
 mouse clones that include the region but has not been detected in rat or human  $\alpha_d$   
 clones. The eighteen base deletion is characterized by a 12 base palindromic  
 sequence AAGCAGGAGCTCCTGTGT (SEQ ID NO: 91). This inverted repeat  
 20 in the nucleic acid sequence is self-complementary and may form a loop out,  
 causing cleavage during reverse transcription. The mouse  $\alpha_d$  sequence which  
 includes the additional 18 bases is set forth in SEQ ID NO: 52; the deduced  
 amino acid sequence is set forth in SEQ ID NO: 53.

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Example 21In situ hybridizations in Mouse

Tissue distribution was then determined for mouse  $\alpha_d$  in order to provide a comparison to that in humans, described in Example 6.

5           A single stranded 200 bp mRNA probe was generated from a DNA template, corresponding to nucleotides 3460 to 3707 in the cytoplasmic tail region of the murine cDNA, by *in vitro* RNA transcription incorporating  $^{35}\text{S}$ -UTP (Amersham).

10           Whole mouse embryos (harvested at days 11-18 after fertilization) and various mouse tissues, including spleen, kidney, liver, intestine, and thymus, were hybridized *in situ* with the radiolabeled single-stranded mRNA probe.

15           Tissues were sectioned at 6  $\mu\text{m}$  thickness, adhered to Vectabond (Vector Laboratories, Inc., Burlingame, CA) coated slides, and stored at  $-70^\circ\text{C}$ . Prior to use, slides were removed from  $-70^\circ\text{C}$  and placed at  $50^\circ\text{C}$  for approximately 5 minutes. Sections were fixed in 4% paraformaldehyde for 20 minutes at  $4^\circ\text{C}$ , dehydrated with an increasing ethanol gradient (70-95-100%) for 1 minute at  $4^\circ\text{C}$  at each concentration, and air dried for 30 minutes at room temperature. Sections were denatured for 2 minutes at  $70^\circ\text{C}$  in 70% formamide/2X SSC, rinsed twice in 2X SSC, dehydrated with the ethanol gradient described *supra* and air dried for 30 minutes. Hybridization was carried out  
20           overnight (12-16 hours) at  $55^\circ\text{C}$  in a solution containing  $^{35}\text{S}$ -labeled riboprobes at  $6 \times 10^5$  cpm/section and diethylpyrocarbonate (DEPC)-treated water to give a final concentration of 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 7.5, 10% dextran sulfate, 1X Denhardt's solution, 100 mM dithiothreitol (DTT) and  
25           5 mM EDTA. After hybridization, sections were washed for 1 hour at room temperature in 4X SSC/10 mM DTT, 40 minutes at  $60^\circ\text{C}$  in 50% formamide/2X SSC/10 mM DTT, 30 minutes at room temperature in 2X SSC, and 30 minutes at room temperature in 0.1X SSC. The sections were dehydrated, air dried for 2 hours, coated with Kodak NTB2 photographic emulsion, air dried for 2 hours,

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developed (after storage at 4°C in complete darkness) and counterstained with hematoxylin/eosin.

Spleen tissue showed a strong signal primarily in the red pulp. This pattern is consistent with that of tissue macrophage distribution in the spleen, but does not exclude other cell types.

### Example 22

#### Generation of Mouse Expression Constructs

In order to construct an expression plasmid including mouse cDNA sequences exhibiting homology to human  $\alpha_d$ , inserts from clones A1160 and B3800 were ligated. Prior to this ligation, however, a 5' leader sequence, including an initiating methionine, was added to clone A1160. A primer designated "5' PCR leader" (SEQ ID NO: 47) was designed to contain: (1) identical nonspecific bases at positions 1-6 allowing for digestion; (2) a *Bam*HI site (underlined in SEQ ID NO: 47) from positions 7-12 to facilitate subcloning into an expression vector; (3) a consensus Kozak sequence from positions 13-18, (4) a signal sequence including a codon for an initiating methionine (bold in SEQ ID NO: 47), and (5) an additional 31 bases of specifically overlapping 5' sequence from clone A1160 to allow primer annealing. A second primer designated "3' end frag" (SEQ ID NO: 48) was used with primer "5' PCR leader" to amplify the insert from clone A1160.

5'-AGTTACGGATCCGGCACCATGAC-  
-CTTCGGCACTGTGATCCTCCTGTGTG-3' (SEQ ID NO: 47)

5'-GCTGGACGATGGCATCCAC-3' (SEQ ID NO: 48)

The resulting PCR product did not digest with *Bam*HI, suggesting that an insufficient number of bases preceded the restriction site, prohibiting

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recognition by the enzyme. The length of the "tail" sequence preceding the *Bam*HI site in the 5' primer (SEQ ID NO: 47) was increased and PCR was repeated on the amplification product from the first PCR. A 5' primer, designated mAD.5'.2 (SEQ ID NO: 49), was designed with additional nonspecific bases at positions 1-4 and an additional 20 bases specifically overlapping the previously employed "5' PCR leader" primer sequences.

5'-GTAGAGTTACGGATCCGGCACCAT-3' (SEQ ID NO: 49)

Primers "mAD.5'.2" and "3' end frag" were used together in PCR with the product from the first amplification as template. A resulting secondary PCR product was subcloned into plasmid pCRtmII (Invitrogen) according to manufacturer's suggested protocol and transformed into competent One shot cells (Invitrogen). One clone containing the PCR product was identified by restriction enzyme analysis using *Bam*HI and *Eco*RI and sequenced. After the sequence was verified, the insert was isolated by digestion with *Bam*HI and *Eco*RI and gel purified.

The insert from clone B3800 was isolated by digestion with *Eco*RI and *Nor*I, gel purified, and added to a ligation reaction which included the augmented A1160 *Bam*HI/*Eco*RI fragment. Ligation was allowed to proceed for 14 hours at 14°C. Vector pcDNA.3 (Invitrogen), digested with *Bam*HI and *Nor*I, was added to the ligation reaction with additional ligase and the reaction was continued for another 12 hours. An aliquot of the reaction mixture was transformed into competent *E. coli* cells, the resulting colonies cultured, and one positive clone identified by PCR analysis with the primers 11.b-1/2FOR1 and 11.b-1/2REV11 (SEQ ID NOS: 50 and 51, respectively). These primers bridge the A1160 and B3800 fragments, therefore detection of an amplification product indicates the two fragments were ligated. The sequence of the positive clone was



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thymidine kinase encoding cassettes. Further analysis of this clone with an I domain probe (corresponding to nucleotides 454-1064 in SEQ ID NO: 45) indicated that the clone did not contain I domain encoding sequences.

5 Using the same I domain probe, the  $\lambda$ FIXII genomic library was rescreened. Initially, six positive clones were detected, one of which remained positive upon secondary screening. DNA isolated from this clone reacted strongly in Southern analysis with an I domain probe. No reactivity was detected using the original 750 bp probe, however, indicating that this clone included regions 5' to nucleotides 1985-2773 of SEQ ID NO: 45..

10 Alternatively, the lack of hybridization to the 750 bp probe may have suggested that the clone was another member of the integrin family of proteins. To determine if this explanation was plausible, the 13 kb insert was subcloned into pBluescript SKII<sup>+</sup>. Purified DNA was sequenced using primers corresponding to  $\alpha_d$  I domain nucleic acid sequences 441-461, 591-612, 717-739, 15 and reverse 898-918 in SEQ ID NO: 52. Sequence information was obtained using only the first 4441-4461 primer, and only the 5'-most exon of the I domain was efficiently amplified. The remainder of the I domain was not amplified. The resulting clone therefore comprised exon 6 of the mouse  $\alpha_d$  gene, and intronic sequences to the 3' and 5' end of the exon. Exon 7 was not represented in the 20 clone. After sequencing, a construct is generated containing neomycin resistance and thymidine kinase genes.

The neomycin resistance ( $neo^r$ ) gene is inserted into the resulting plasmid in a manner that interrupts the protein coding sequence of the genomic mouse DNA. The resulting plasmid therefore contains a  $neo^r$  gene within the 25 mouse genomic DNA sequences, all of which are positioned within a thymidine kinase encoding region. Plasmid construction in this manner is required to favor homologous recombination over random recombination [Chisaka, *et al.*, *Nature* 355:516-520 (1992)].

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verified with the primers set out in SEQ ID NOS: 50 and 51, which amplify from base 100 to 1405 after the initiating methionine.

### Example 23

#### Construction of a Knock-out Mouse

5           In order to more accurately assess the immunological role of the protein encoded by the putative mouse  $\alpha_d$  cDNA, a "knock-out" mouse is designed wherein the genomic DNA sequence encoding the putative  $\alpha_d$  homolog is disrupted by homologous recombination. The significance of the protein encoded by the disrupted gene is thereby assessed by the absence of the encoded  
10           protein. Generation of "knock-out" mice is described in Deng, *et al.*, *Mol. Cell. Biol.* 13:2134-2140 (1993).

          Design of such a mouse begins with construction of a plasmid containing sequences to be "knocked out" by homologous recombination events. A 750 base pair fragment of the mouse cDNA (corresponding to nucleotides 1985  
15           to 2733 in SEQ ID NO: 45) was used to identify a mouse genomic sequence encoding the putative mouse  $\alpha_d$  homolog from a  $\lambda$ FIXII genomic library. Primary screening resulted in 14 positive plaques, seven of which were confirmed by secondary screening. Liquid lysates were obtained from two of the plaques giving the strongest signal and the  $\lambda$  DNA was isolated by conventional methods.  
20           Restriction mapping and Southern analysis confirmed the authenticity of one clone, designated 14-1, and the insert DNA was isolated by digestion with *NotI*. This fragment was cloned into Bluescript SKII<sup>+</sup>.

          In order to identify a restriction fragment of approximately 9 to 14 kb, a length reported to optimize the probability of homologous recombination events, Southern hybridization was performed with the 750 bp cDNA probe.  
25           Prior to hybridization, a restriction map was constructed for clone 14-1. A 12 kb fragment was identified as a possible candidate and this fragment was subcloned into pBluescript SKII<sup>+</sup> in a position wherein the mouse DNA is flanked by

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$10^6$  dpm/ml. Hybridization was carried out at 42°C for 16-18 hours. Filters were washed extensively in 2X SSPE/0.1% SDS at room temperature and exposed to X-ray film to visualize any hybridizing plaques.

Two clones with significant sequence homology to human  $\alpha_d$  were identified. Clone #2 was approximately 800 bp in length and mapped to the 5' end of human  $\alpha_d$ . Clone #2 includes an initiating methionine and complete leader sequence. Clone #7 was approximately 1.5 kb and includes an initiating methionine. The 5' end of clone #7 overlapped that of clone #2, while the 3' sequences terminated at a point beyond the I domain sequences. Internal sequencing of clone #7 is performed using the nested deletions sequencing technique.

The predicted N terminal amino acid sequence for rabbit  $\alpha_d$  as determined from clones #2 and #7 indicated a protein with 73% identity with human  $\alpha_d$ , 65% identity with mouse  $\alpha_d$ , and 58% identity with mouse CD11b, human CD11b, and human CD11c. The nucleic acid sequence for clone #2 is set out in SEQ ID NO: 92; the predicted amino acid sequence is set out in SEQ ID NO: 93

Isolation of a full length rabbit  $\alpha_d$  cDNA is carried out using labeled rabbit fragment, clone # 7, and rescreening the cDNA library from which the fragment was derived.

Isolation of a rabbit  $\alpha_d$  clone allows expression of the protein, either on the surface of transfectants or as a soluble full length or truncated form. This protein is then used as an immunogen for the production of monoclonal antibodies for use in rabbit models of human disease states.

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**Example 24****Cloning of Rabbit  $\alpha_d$  - Construction and Screening of the Rabbit cDNA Library**

Identification of human  $\alpha_d$  homologs in rats and mice led to the investigation of the existence of a rabbit homolog which would be useful in rabbit models of human disease states described *infra*.

Poly A<sup>+</sup> RNA was prepared from a whole rabbit spleen using an Invitrogen FastTrack kit (San Diego, CA) according to manufacturer's suggested protocol and reagents supplied with the kit. From 1.65 g tissue, 73  $\mu$ g poly A<sup>+</sup> RNA were isolated. The rabbit spleen RNA was used to construct a ZAP Express cDNA library using a kit from Stratagene (La Jolla, CA). Resulting cDNA was directionally cloned into *Eco*RI and *Xho*I sites in the lambda arms of a pBK-CMV phagemid vector. Gigapack II Gold (Stratagene) was used to package the lambda arms into phage particles. The resulting library titer was estimated to be approximately  $8 \times 10^5$  particles, with an average insert size of 1.2 kb.

The library was amplified once by plating for confluent plaque growth and cell lysate was collected. The amplified library was plated at approximately 30,000 plaque forming units (pfu) per 150 mm plate with *E. coli* and the resulting mixture incubated for 12-16 hrs at 37°C to allow plaque formation. Phage DNA was transferred onto Hybond N<sup>+</sup> nylon membranes (Amersham, Arlington Heights, Illinois). The membranes were hybridized with a mixture of two random primed radiolabeled mouse  $\alpha_d$  PCR DNA probes. The first probe was generated from a PCR product spanning nucleotides 149-946 in SEQ ID NO: 52. The second probe was from a PCR product spanning nucleotides 2752-3651 in SEQ ID NO: 52. Probes were labeled by random priming (Boehringer Mannheim Random Primed DNA Labeling Kit) and the reaction mixture was passed over a Sephadex G-50 column to remove unincorporated nucleotides. The hybridization solution was composed of 5X SSPE, 5X Denhardt's, 1% SDS, 40% Formamide and the labeled probes at 1 x

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functions which damage normal host tissue through either specific autoimmune responses or as a result of bystander cell damage.

Disease states in which there is evidence of macrophages playing a significant role in the disease process include multiple sclerosis, arthritis, graft atherosclerosis, some forms of diabetes and inflammatory bowel disease. Animal models, discussed below, have been shown to reproduce many of the aspects of these human disorders. Inhibitors of  $\alpha_d$  function are tested in these model systems to determine if the potential exists for treating the corresponding human diseases.

#### 10 A. Graft Arteriosclerosis

Cardiac transplantation is now the accepted form of therapeutic intervention for some types of end-state heart disease. As the use of cyclosporin A has increased one year survival rates to 80%, the development of progressive graft arteriosclerosis has emerged as the leading cause of death in cardiac transplants surviving beyond the first year. Recent studies have found that the incidence of significant graft arteriosclerosis 3 years following a cardiac transplant is in the range of 36-44% [Adams, *et al.*, *Transplantation* 53:1115-1119 (1992); Adams, *et al.*, *Transplantation* 56:794-799 (1993)].

Graft arteriosclerosis typically consists of diffuse, occlusive, intimal lesions which affect the entire coronary vessel wall, and are often accompanied by lipid deposition. While the pathogenesis of graft arteriosclerosis remains unknown, it is presumably linked to histocompatibility differences between donor and recipient, and is immunologic in nature. Histologically, the areas of intimal thickening are composed primarily of macrophages, although T cells are occasionally seen. It is therefore possible that macrophages expressing  $\alpha_d$  may play a significant role in the induction and/or development of graft arteriosclerosis. In such a case, monoclonal antibodies or small molecule inhibitors (for example, soluble ICAM-R) of  $\alpha_d$  function could be given

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**Example 25****Animal Models For Determining  $\alpha_d$  Therapeutic Utility**

Immunohistologic data in dog and *in situ* hybridization in rats and mice has determined that in spleen  $\alpha_d$  is expressed primarily by macrophages present in red pulp and in lymph nodes,  $\alpha_d$  is found in medullary cords and sinuses. The expression pattern is remarkably similar to what has been reported for two murine antigens defined by the monoclonal antibodies F4/80 and SK39. While biochemical characterization of these murine antigens has demonstrated that they are distinct from  $\alpha_d$ , it is highly probable that  $\alpha_d$  defines the same macrophage subset as the murine F4/80 and SK39 antigens.

In mouse, SK39-positive macrophages have been identified in splenic red pulp where they may participate in the clearance of foreign materials from circulation, and in medulla of lymph nodes [Jutila, *et al.*, *J. Leukocyte Biol.* 54:30-39 (1993)]. SK39-positive macrophages have also been reported at sites of both acute and chronic inflammation. Furthermore, monocytes recruited to thioglycolate-inflamed peritoneal cavities also express the SK39 antigen. Collectively, these findings suggest that, if SK39<sup>+</sup> cells are also  $\alpha_d$ <sup>+</sup>, then these cells are responsible for the clearance of foreign materials in the spleen and participate in inflammation where macrophages play a significant role.

While the function of  $\alpha_d$  remains unclear, other more well characterized  $\beta_2$  integrins have been shown to participate in a wide variety of adhesion events that facilitate cell migration, enhance phagocytosis, and promote cell-cell interactions, events which all lead to upregulation of inflammatory processes. Therefore, it is highly plausible that interfering with the normal  $\alpha_d$  function may also interfere with inflammation where macrophages play a significant role. Such an anti-inflammatory effect could result from: i) blocking macrophage recruitment to sites of inflammation, ii) preventing macrophage activation at the site of inflammation or iii) interfering with macrophage effector

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luminal surface of the ascending aorta [Rosenfeld, *et al.*, *Arteriosclerosis* 7:9-23 (1987); Rosenfeld, *et al.*, *Arteriosclerosis* 7:24-34 (1987)]. The atherosclerotic lesions seen in these rabbits are similar to those in humans. Lesions contain large numbers of T cells, most of which express CD45RO, a marker associated with memory T cells. Approximately half of the infiltrating T cells also express MHC class II antigen and some express the IL-2 receptor suggesting that many of the cells are in an activated state.

One feature of the atherosclerotic lesions found in cholesterol fed rabbits, but apparently absent in rodent models, is the accumulation of foam cell-rich lesions. Foam cell macrophages are believed to result from the uptake of oxidized low-density lipoprotein (LDL) by specific receptors. Oxidized LDL particles have been found to be toxic for some cell types including endothelial cells and smooth muscle cells. The uptake of potentially toxic, oxidized LDL particles by macrophages serves as an irritant and drives macrophage activation, contributing to the inflammation associated with atherosclerotic lesions.

Once monoclonal antibodies have been generated to rabbit  $\alpha_d$ , cholesterol fed rabbits are treated. Treatments include prophylactic administration of  $\alpha_d$  monoclonal antibodies or small molecule inhibitors, to demonstrate that  $\alpha_d^+$  macrophages are involved in the disease process. Additional studies would demonstrate that monoclonal antibodies to  $\alpha_d$  or small molecule inhibitors are capable of reversing vessel damage detected in rabbits fed an atherogenic diet.

### C. Insulin-dependent Diabetes

BB rats spontaneously develop insulin-dependent diabetes at 70-150 days of age. Using immunohistochemistry, MHC class II<sup>+</sup>, ED1<sup>+</sup> macrophages can be detected infiltrating the islets early in the disease. Many of the macrophages appear to be engaged in phagocytosis of cell debris or normal cells. As the disease progresses, larger numbers of macrophages are found infiltrating

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prophylactically to individuals who received heart transplants and are at risk of developing graft arteriosclerosis.

Although atherosclerosis in heart transplants presents the greatest threat to life, graft arteriosclerosis is also seen in other solid organ transplants, including kidneys and livers. Therapeutic use of  $\alpha_d$  blocking agents could prevent graft arteriosclerosis in other organ transplants and reduce complications resulting from graft failure.

One model for graft arteriosclerosis in the rat involves heterotopic cardiac allografts transplanted across minor histocompatibility barriers. When Lewis cardiac allografts are transplanted into MHC class I and II compatible F-344 recipients, 80% of the allografts survive at least 3 weeks, while 25% of the grafts survive indefinitely. During this low-grade graft rejection, arteriosclerosis lesions form in the donor heart. Arterial lesions in 120 day old allografts typically have diffuse fibrotic intimal thickening indistinguishable in appearance from graft arteriosclerosis lesions found in rejecting human cardiac allografts.

Rats are transplanted with hearts mismatched at minor histocompatibility antigens, for example Lewis into F-344. Monoclonal antibodies specific for rat  $\alpha_d$  or small molecule inhibitors of  $\alpha_d$  are given periodically to transplant recipients. Treatment is expected to reduce the incidence of graft arteriosclerosis in non-rejecting donor hearts. Treatment of rats with  $\alpha_d$  monoclonal antibodies or small molecule inhibitors may not be limited to prophylactic treatments. Blocking  $\alpha_d$  function is also expected to reduce macrophage mediated inflammation and allow reversal of arterial damage in the graft.

#### B. Atherosclerosis in Rabbits Fed Cholesterol

Rabbits fed an atherogenic diet containing a cholesterol supplement for approximately 12-16 weeks develop intimal lesions that cover most of the



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the islets, although significant numbers of T cells, and later B cells, also appear to be recruited to the site [Hanenberg, *et al.*, *Diabetologia* 32:126-134 (1989)].

Development of diabetes in BB rats appears to depend on both early macrophage infiltration and subsequent T cells recruitment. Treatment of BB rats with silica particles, which are toxic to macrophages, has been effective in blocking the early macrophage infiltration of the islets. In the absence of early macrophage infiltration, subsequent tissue damage by an autoaggressive lymphocyte population fails to occur. Administration of monoclonal antibody OX-19 (specific for rat CD5) or monoclonal antibody OX-8 (specific for rat CD8), which block the T cell-associated phase of the disease, is also effective in suppressing the development of diabetes.

The central role of macrophages in the pathology of this model makes it attractive for testing inhibitors of  $\alpha_d$  function. Rats genetically predisposed to the development of insulin-dependent diabetes are treated with monoclonal antibodies to  $\alpha_d$  or small molecule inhibitors and evaluated for the development of the disease. Preventing or delaying clinical onset is evidence that  $\alpha_d$  plays a pivotal role in macrophage damage to the islet cells.

#### D. Inflammatory Bowel Disease (Crohn's Disease, Ulcerative Colitis)

Animal models used in the study of inflammatory bowel disease (IBD) are generally elicited by intrarectal administration of noxious irritants (e.g. acetic acid or trinitrobenzene sulfonic acid/ethanol). Colonic inflammation induced by these agents is the result of chemical or metabolic injury and lacks the chronic and spontaneously relapsing inflammation associated with human IBD. However, a recently described model using subserosal injections of purified peptidoglycan-polysaccharide (PG-PS) polymers from either group A or group D streptococci appears to be a more physiologically relevant model for human IBD [Yamada, *et al.*, *Gastroenterology* 104:759-771 (1993)].

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In this model PG-PS is injected into the subserosal layer of the distal colon. The resulting inflammatory response is biphasic with an initial acute episode three days after injection, which is followed by a spontaneous chronic phase three to four weeks later. The late phase response is granulomatous in nature, and results in colonic thickening, adhesions, colonic nodules and mucosal lesions. In addition to mucosal injury, PG-PS colitis frequently leads to arthritis anemia and granulomatous hepatitis. The extraintestinal manifestations of the disease make the model attractive for studying Crohn's colitis in that a significant number of patients with active Crohn's disease suffer from arthritic joint disease and hepatobiliary inflammation.

Granulomatous lesions are the result of chronic inflammation which leads to the recruitment and subsequent activation of cells of the monocyte/macrophage lineage. Presence of granulomatous lesions in Crohn's disease and the above animal model make this an attractive clinical target for  $\alpha_d$  monoclonal antibodies or other inhibitors of  $\alpha_d$  function. Inhibitors of  $\alpha_d$  function are expected to block the formation of lesions associated with IBD or even reverse tissue damage seen in the disease.

#### E. Arthritis

Arthritis appears to be a multi-factorial disease process involving a variety of inflammatory cell types including neutrophils, T lymphocytes and phagocytic macrophages. Although a variety of arthritis models exist, preparations of streptococcal cell wall proteoglycan produce a disorder most similar to the human disease.

In rats, streptococcal cell wall induces inflammation of peripheral joints characterized by repeated episodes of disease progression followed by remission and eventually resulting in joint destruction over a period of several months [Cromartie, *et al.*, *J.Exp.Med.* 146:1585-1602 (1977); Schwab *et al.*, *Infection and Immunity* 59:4436-4442 (1991)]. During the chronic phase of the

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disease, mononuclear phagocytes or macrophages are believed to play a major role in destruction of the synovium. Furthermore, agents which suppress the recruitment of macrophages into the synovium effectively reduce the inflammation and pathology characteristic of arthritis.

5                   A central role for the macrophage in synovium destruction that leads to arthritis predicts that monoclonal antibodies to  $\alpha_d$  or inhibitors of  $\alpha_d$  function may have therapeutic potential in the treatment of this disease. As in other models previously described,  $\alpha_d$  monoclonal antibodies or small molecule inhibitors administered prophylactically are expected to block or moderate joint  
10 inflammation and prevent destruction of the synovium. Agents that interfere with  $\alpha_d$  function may also moderate ongoing inflammation by preventing the recruitment of additional macrophages to the joint or blocking macrophage activation. The net result would be to reverse ongoing destruction of the joint and facilitate tissue repair.

#### 15                   F. Multiple Sclerosis

Although pathogenesis of multiple sclerosis (MS) remains unclear, it is generally accepted that the disease is mediated by  $CD4^+$  T cells which recognize autoantigens in the central nervous system and initiate an inflammatory cascade. The resulting immune response results in the recruitment of additional  
20 inflammatory cells, including activated macrophages which contribute to the disease. Experimental autoimmune encephalomyelitis (EAE) is an animal model which reproduces some aspects of MS. Recently, monoclonal antibodies reactive with CD11b/CD18 [Huitinga, *et al.*, *Eur.J.Immunol.* 23:709-715 (1993)] present on inflammatory macrophages have been shown to block both clinical and  
25 histologic disease. The results suggest that monoclonal antibodies or small molecule inhibitors to  $\alpha_d$  are likely to be effective in blocking the inflammatory response in EAE. Such agents also have important therapeutic applications in the treatment of MS.

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Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

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THE INTERNATIONAL APPLICATION**

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## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 3..3485

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCC ACC AAC GGC TCC CGG CTC CTG GCC TGT GGC CCG ACC CTG CAC AGA	335
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100 105 110	
GTC TGT GGG GAG AAC TCA TAC TCA AAG GGT TCC TGC CTC CTG CTG GGC	383
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210 215 220	

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CCC CAG GCA GAG AAG GCT GGC ATC ATC CGC TAC GCT ATC GGG GTG GGA Pro Gln Ala Glu Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly Val Gly 275 280 285	863
CAC GCT TTC CAG GCA CCC ACT GCC AGG CAG GAG CTG AAT ACC ATC AGC His Ala Phe Gln Gly Pro Thr Ala Arg Gln Glu Leu Asn Thr Ile Ser 290 295 300	911
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CTT GGC AGC ATC CAG AAG CAG CTG CAG GAG AAG ATC TAT GCA GTT GAG Leu Gly Ser Ile Gln Lys Gln Leu Gln Glu Lys Ile Tyr Ala Val Glu 320 325 330 335	1007
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GCC ACA CTG TAC AAG CTT GGC TTC TTC AAA CGC CAC TAC AAG GAA ATG Ala Thr Leu Tyr Lys Leu Gly Phe Phe Lys Arg His Tyr Lys Glu Met 1120 1125 1130 1135	3407
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- 87 -

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3726

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- (A) LENGTH: 1161 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gly Phe Gly Gln Ser Val Val Gln Phe Gly Gly Ser Arg Leu Val Val
      35           40           45
Gly Ala Pro Leu Glu Val Val Ala Ala Asn Gln Thr Gly Arg Leu Tyr
      50           55           60
Asp Cys Ala Ala Ala Thr Gly Met Cys Gln Pro Ile Fro Leu His Ile
      65           70           75           80
Arg Pro Glu Ala Val Asn Met Ser Leu Gly Leu Thr Leu Ala Ala Ser
      85           90           95
Thr Asn Gly Ser Arg Leu Leu Ala Cys Gly Pro Thr Leu His Arg Val
      100          105          110
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Phe Thr Ala Thr Gly Ile Leu Thr Val Val Thr Gln Leu Phe His His
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Lys Asn Gly Ala Arg Lys Ser Ala Lys Lys Ile Leu Ile Val Ile Thr
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- 88 -

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 Ala Phe Gln Gly Pro Thr Ala Arg Gln Glu Leu Asn Thr Ile Ser Ser  
 290 295 300  
 Ala Pro Pro Gln Asp His Val Phe Lys Val Asp Asn Phe Ala Ala Leu  
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 Gly Ser Ile Gln Lys Gln Leu Gln Glu Lys Ile Tyr Ala Val Glu Gly  
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 Ser Gly Ile Ser Pro Ser His Ser Gln Arg Ile Ala Ser Ser Gln Leu  
 565 570 575

- 89 -

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 850 855 860  
 Pro Ile Phe His Glu Gly Ser Asn Gly Thr Phe Ile Val Thr Phe Asp  
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 Val Ser Tyr Lys Ala Thr Leu Gly Asp Arg Met Leu Met Arg Ala Ser  
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- 90 -

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 Leu Glu Leu Pro Val Lys Tyr Ala Val Tyr Thr Met Ile Ser Arg Gln  
                     915                    920                    925  
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                     1060                    1065                    1070  
 Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala Phe Met Arg Ala Gln Met  
                     1075                    1080                    1085  
 Glu Met Val Leu Glu Glu Asp Glu Val Tyr Asn Ala Ile Pro Ile Ile  
                     1090                    1095                    1100  
 Met Gly Ser Ser Val Gly Ala Leu Leu Leu Leu Ala Leu Ile Thr Ala  
                     1105                    1110                    1115                    1120  
 Thr Leu Tyr Lys Leu Gly Phe Phe Lys Arg His Tyr Lys Glu Met Leu  
                     1125                    1130                    1135  
 Glu Asp Lys Pro Glu Asp Thr Ala Thr Phe Ser Gly Asp Asp Phe Ser  
                     1140                    1145                    1150  
 Cys Val Ala Pro Asn Val Pro Lys Ser  
                     1155                    1160

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1153 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Ala	Leu	Arg	Val	Leu	Leu	Leu	Thr	Ala	Leu	Thr	Leu	Cys	His	Gly	1	5	10	15
Phe	Asn	Leu	Asp	Thr	Glu	Asn	Ala	Met	Thr	Phe	Gln	Glu	Asn	Ala	Arg	20	25	30	
Gly	Phe	Gly	Gln	Ser	Val	Val	Gln	Leu	Gln	Gly	Ser	Arg	Val	Val	Val	35	40	45	
Gly	Ala	Pro	Gln	Glu	Ile	Val	Ala	Ala	Asn	Gln	Arg	Gly	Ser	Leu	Tyr	50	55	60	
Gln	Cys	Asp	Tyr	Ser	Thr	Gly	Ser	Cys	Glu	Pro	Ile	Arg	Leu	Gln	Val	65	70	75	80
Pro	Val	Glu	Ala	Val	Asn	Met	Ser	Leu	Gly	Leu	Ser	Leu	Ala	Ala	Thr	85	90	95	
Thr	Ser	Pro	Pro	Gln	Leu	Leu	Ala	Cys	Gly	Pro	Thr	Val	His	Gln	Thr	100	105	110	
Cys	Ser	Glu	Asn	Thr	Tyr	Val	Lys	Gly	Leu	Cys	Phe	Leu	Phe	Gly	Ser	115	120	125	
Asn	Leu	Arg	Gln	Gln	Pro	Gln	Lys	Phe	Pro	Glu	Ala	Leu	Arg	Gly	Cys	130	135	140	
Pro	Gln	Glu	Asp	Ser	Asp	Ile	Ala	Phe	Leu	Ile	Asp	Gly	Ser	Gly	Ser	145	150	155	160
Ile	Ile	Pro	His	Asp	Phe	Arg	Arg	Met	Lys	Glu	Phe	Val	Ser	Thr	Val	165	170	175	
Met	Glu	Gln	Leu	Lys	Lys	Ser	Lys	Thr	Leu	Phe	Ser	Leu	Met	Gln	Tyr	180	185	190	
Ser	Glu	Glu	Phe	Arg	Ile	His	Phe	Thr	Phe	Lys	Glu	Phe	Gln	Asn	Asn	195	200	205	
Pro	Asn	Pro	Arg	Ser	Leu	Val	Lys	Pro	Ile	Thr	Gln	Leu	Leu	Gly	Arg	210	215	220	
Thr	His	Thr	Ala	Thr	Gly	Ile	Arg	Lys	Val	Val	Arg	Glu	Leu	Phe	Asn	225	230	235	240
Ile	Thr	Asn	Gly	Ala	Arg	Lys	Asn	Ala	Phe	Lys	Ile	Leu	Val	Val	Ile	245	250	255	
Thr	Asp	Gly	Glu	Lys	Phe	Gly	Asp	Pro	Leu	Gly	Tyr	Glu	Asp	Val	Ile	260	265	270	
Pro	Glu	Ala	Asp	Arg	Glu	Gly	Val	Ile	Arg	Tyr	Val	Ile	Gly	Val	Gly	275	280	285	
Asp	Ala	Phe	Arg	Ser	Glu	Lys	Ser	Arg	Gln	Glu	Leu	Asn	Thr	Ile	Ala	290	295	300	

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Ser Lys Pro Pro Arg Asp His Val Phe Gln Val Asn Asn Phe Glu Ala  
 305 310 315 320  
 Leu Lys Thr Ile Gln Asn Gln Leu Arg Glu Lys Ile Phe Ala Ile Glu  
 325 330 335  
 Gly Thr Gln Thr Gly Ser Ser Ser Ser Phe Glu His Glu Met Ser Gln  
 340 345 350  
 Glu Gly Phe Ser Ala Ala Ile Thr Ser Asn Gly Pro Leu Leu Ser Thr  
 355 360 365  
 Val Gly Ser Tyr Asp Trp Ala Gly Gly Val Phe Leu Tyr Thr Ser Lys  
 370 375 380  
 Glu Lys Ser Thr Phe Ile Asn Met Thr Arg Val Asp Ser Asp Met Asn  
 385 390 395 400  
 Asp Ala Tyr Leu Gly Tyr Ala Ala Ala Ile Ile Leu Arg Asn Arg Val  
 405 410 415  
 Gln Ser Leu Val Leu Gly Ala Pro Arg Tyr Gln His Ile Gly Leu Val  
 420 425 430  
 Ala Met Phe Arg Gln Asn Thr Gly Met Trp Glu Ser Asn Ala Asn Val  
 435 440 445  
 Lys Gly Thr Gln Ile Gly Ala Tyr Phe Gly Ala Ser Leu Cys Ser Val  
 450 455 460  
 Asp Val Asp Ser Asn Gly Ser Thr Asp Leu Val Leu Ile Gly Ala Pro  
 465 470 475 480  
 His Tyr Tyr Glu Gln Thr Arg Gly Gly Gln Val Ser Val Cys Pro Leu  
 485 490 495  
 Pro Arg Gly Gln Arg Ala Arg Trp Gln Cys Asp Ala Val Leu Tyr Gly  
 500 505 510  
 Glu Gln Gly Gln Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val Leu  
 515 520 525  
 Gly Asp Val Asn Gly Asp Lys Leu Thr Asp Val Ala Ile Gly Ala Pro  
 530 535 540  
 Gly Glu Glu Asp Asn Arg Gly Ala Val Tyr Leu Phe His Gly Thr Ser  
 545 550 555 560  
 Gly Ser Gly Ile Ser Pro Ser His Ser Gln Arg Ile Ala Gly Ser Lys  
 565 570 575  
 Leu Ser Pro Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly Gln  
 580 585 590  
 Asp Leu Thr Met Asp Gly Leu Val Asp Leu Thr Val Gly Ala Gln Gly  
 595 600 605  
 His Val Leu Leu Leu Arg Ser Gln Pro Val Leu Arg Val Lys Ala Ile  
 610 615 620



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Met Glu Phe Asn Pro Arg Glu Val Ala Arg Asn Val Phe Glu Cys Asn  
 625 630 635 640  
 Asp Gln Val Val Lys Gly Lys Glu Ala Gly Glu Val Arg Val Cys Leu  
 645 650 655  
 His Val Gln Lys Ser Thr Arg Asp Arg Leu Arg Glu Gly Gln Ile Gln  
 660 665 670  
 Ser Val Val Thr Tyr Asp Leu Ala Leu Asp Ser Gly Arg Pro His Ser  
 675 680 685  
 Arg Ala Val Phe Asn Glu Thr Lys Asn Ser Thr Arg Arg Gln Thr Gln  
 690 695 700  
 Val Leu Gly Leu Thr Gln Thr Cys Glu Thr Leu Lys Leu Gln Leu Pro  
 705 710 715 720  
 Asn Cys Ile Glu Asp Pro Val Ser Pro Ile Val Leu Arg Leu Asn Phe  
 725 730 735  
 Ser Leu Val Gly Thr Pro Leu Ser Ala Phe Gly Asn Leu Arg Pro Val  
 740 745 750  
 Leu Ala Glu Asp Ala Gln Arg Leu Phe Thr Ala Leu Phe Pro Phe Glu  
 755 760 765  
 Lys Asn Cys Gly Asn Asp Asn Ile Cys Gln Asp Asp Leu Ser Ile Thr  
 770 775 780  
 Phe Ser Phe Met Ser Leu Asp Cys Leu Val Val Gly Gly Pro Arg Glu  
 785 790 795 800  
 Phe Asn Val Thr Val Thr Val Arg Asn Asp Gly Glu Asp Ser Tyr Arg  
 805 810 815  
 Thr Gln Val Thr Phe Phe Phe Pro Leu Asp Leu Ser Tyr Arg Lys Val  
 820 825 830  
 Ser Thr Leu Gln Asn Gln Arg Ser Gln Arg Ser Trp Arg Leu Ala Cys  
 835 840 845  
 Glu Ser Ala Ser Ser Thr Glu Val Ser Gly Ala Leu Lys Ser Thr Ser  
 850 855 860  
 Cys Ser Ile Asn His Pro Ile Phe Pro Glu Asn Ser Glu Val Thr Phe  
 865 870 875 880  
 Asn Ile Thr Phe Asp Val Asp Ser Lys Ala Ser Leu Gly Asn Lys Leu  
 885 890 895  
 Leu Leu Lys Ala Asn Val Thr Ser Glu Asn Asn Met Pro Arg Thr Asn  
 900 905 910  
 Lys Thr Glu Phe Gln Leu Glu Leu Pro Val Lys Tyr Ala Val Tyr Met  
 915 920 925  
 Val Val Thr Ser His Gly Val Ser Thr Lys Tyr Leu Asn Phe Thr Ala  
 930 935 940

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Ser Glu Asn Thr Ser Arg Val Met Gln His Gln Tyr Gln Val Ser Asn  
 945 950 955 960  
 Leu Gly Gln Arg Ser Leu Pro Ile Ser Leu Val Phe Leu Val Pro Val  
 965 970 975  
 Arg Leu Asn Gln Thr Val Ile Trp Asp Arg Pro Gln Val Thr Phe Ser  
 980 985 990  
 Glu Asn Leu Ser Ser Thr Cys His Thr Lys Glu Arg Leu Pro Ser His  
 995 1000 1005  
 Ser Asp Phe Leu Ala Glu Leu Arg Lys Ala Pro Val Val Asn Cys Ser  
 1010 1015 1020  
 Ile Ala Val Cys Gln Arg Ile Gln Cys Asp Ile Pro Phe Phe Gly Ile  
 1025 1030 1035 1040  
 Gln Glu Glu Phe Asn Ala Thr Leu Lys Gly Asn Leu Ser Phe Asp Trp  
 1045 1050 1055  
 Tyr Ile Lys Thr Ser His Asn His Leu Leu Ile Val Ser Thr Ala Glu  
 1060 1065 1070  
 Ile Leu Phe Asn Asp Ser Val Phe Thr Leu Leu Pro Gly Gln Gly Ala  
 1075 1080 1085  
 Phe Val Arg Ser Gln Thr Glu Thr Lys Val Glu Pro Phe Glu Val Pro  
 1090 1095 1100  
 Asn Pro Leu Pro Leu Ile Val Gly Ser Ser Val Gly Gly Leu Leu Leu  
 1105 1110 1115 1120  
 Leu Ala Leu Ile Thr Ala Ala Leu Tyr Lys Leu Gly Phe Phe Lys Arg  
 1125 1130 1135  
 Gln Tyr Lys Asp Met Met Ser Glu Gly Gly Pro Pro Gly Ala Glu Pro  
 1140 1145 1150  
 Gln

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1163 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Arg Thr Arg Ala Ala Leu Leu Phe Thr Ala Leu Ala Thr  
 1 5 10 15  
 Ser Leu Gly Phe Asn Leu Asp Thr Glu Glu Leu Thr Ala Phe Arg Val  
 20 25 30

Asp	Ser	Ala	Gly	Phe	Gly	Asp	Ser	Val	Val	Gln	Tyr	Ala	Asn	Ser	Trp
35						40						45			
Val	Val	Val	Gly	Ala	Pro	Gln	Lys	Ile	Ile	Ala	Ala	Asn	Gln	Ile	Gly
50						55				60					
Gly	Leu	Tyr	Gln	Cys	Gly	Tyr	Ser	Thr	Gly	Ala	Cys	Glu	Pro	Ile	Gly
65					70				75					80	
Leu	Gln	Val	Pro	Pro	Glu	Ala	Val	Asn	Met	Ser	Leu	Gly	Leu	Ser	Leu
			85						90					95	
Ala	Ser	Thr	Thr	Ser	Pro	Ser	Gln	Leu	Leu	Ala	Cys	Gly	Pro	Thr	Val
			100					105					110		
His	His	Glu	Cys	Gly	Arg	Asn	Met	Tyr	Leu	Thr	Gly	Leu	Cys	Phe	Leu
		115					120					125			
Leu	Gly	Pro	Thr	Gln	Leu	Thr	Gln	Arg	Leu	Pro	Val	Ser	Arg	Gln	Glu
		130				135					140				
Cys	Pro	Arg	Gln	Glu	Gln	Asp	Ile	Val	Phe	Leu	Ile	Asp	Gly	Ser	Gly
145					150					155					160
Ser	Ile	Ser	Ser	Arg	Asn	Phe	Ala	Thr	Met	Met	Asn	Phe	Val	Arg	Ala
				165					170					175	
Val	Ile	Ser	Gln	Phe	Gln	Arg	Pro	Ser	Thr	Gln	Phe	Ser	Leu	Met	Gln
			180					185					190		
Phe	Ser	Asn	Lys	Phe	Gln	Thr	His	Phe	Thr	Phe	Glu	Glu	Phe	Arg	Arg
		195					200					205			
Thr	Ser	Asn	Pro	Leu	Ser	Leu	Leu	Ala	Ser	Val	His	Gln	Leu	Gln	Gly
		210				215					220				
Phe	Thr	Tyr	Thr	Ala	Thr	Ala	Ile	Gln	Asn	Val	Val	His	Arg	Leu	Phe
225					230					235					240
His	Ala	Ser	Tyr	Gly	Ala	Arg	Arg	Asp	Ala	Ile	Lys	Ile	Leu	Ile	Val
				245					250					255	
Ile	Thr	Asp	Gly	Lys	Lys	Glu	Gly	Asp	Ser	Leu	Asp	Tyr	Lys	Asp	Val
			260					265					270		
Ile	Pro	Met	Ala	Asp	Ala	Ala	Gly	Ile	Ile	Arg	Tyr	Ala	Ile	Gly	Val
		275					280					285			
Gly	Leu	Ala	Phe	Gln	Asn	Arg	Asn	Ser	Trp	Lys	Glu	Leu	Asn	Asp	Ile
		290				295					300				
Ala	Ser	Lys	Pro	Ser	Gln	Glu	His	Ile	Phe	Lys	Val	Glu	Asp	Phe	Asp
305					310					315					320
Ala	Leu	Lys	Asp	Ile	Gln	Asn	Gln	Leu	Lys	Glu	Lys	Ile	Phe	Ala	Ile
				325					330					335	
Glu	Gly	Thr	Glu	Thr	Ile	Ser	Ser	Ser	Ser	Phe	Glu	Leu	Glu	Met	Ala
			340					345					350		

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Gln Glu Gly Phe Ser Ala Val Phe Thr Pro Asp Gly Pro Val Leu Gly  
 355 360 365  
 Ala Val Gly Ser Phe Thr Trp Ser Gly Gly Ala Phe Leu Tyr Pro Pro  
 370 375 380  
 Asn Met Ser Pro Thr Phe Ile Asn Met Ser Gln Glu Asn Val Asp Met  
 385 390 395 400  
 Arg Asp Ser Tyr Leu Gly Tyr Ser Thr Glu Leu Ala Leu Trp Lys Gly  
 405 410 415  
 Val Gln Ser Leu Val Leu Gly Ala Pro Arg Tyr Gln His Ile Gly Lys  
 420 425 430  
 Ala Val Ile Phe Ile Gln Val Ser Arg Gln Trp Arg Met Lys Ala Glu  
 435 440 445  
 Val Ile Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys Ser  
 450 455 460  
 Val Asp Val Asp Thr Asp Gly Ser Thr Asp Leu Val Leu Ile Gly Ala  
 465 470 475 480  
 Pro His Tyr Tyr Glu Gln Thr Arg Gly Gly Gln Val Ser Val Cys Pro  
 485 490 495  
 Leu Pro Arg Gly Trp Arg Arg Trp Trp Cys Asp Ala Val Leu Tyr Gly  
 500 505 510  
 Glu Gln Gly His Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val Leu  
 515 520 525  
 Gly Asp Val Asn Gly Asp Lys Leu Thr Asp Val Val Ile Gly Ala Pro  
 530 535 540  
 Gly Glu Glu Glu Asn Arg Gly Ala Val Tyr Leu Phe His Gly Val Leu  
 545 550 555 560  
 Gly Pro Ser Ile Ser Pro Ser His Ser Gln Arg Ile Ala Gly Ser Gln  
 565 570 575  
 Leu Ser Ser Arg Leu Gln Tyr Phe Gly Gln Ala Leu Ser Gly Gly Gln  
 580 585 590  
 Asp Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Arg Gly  
 595 600 605  
 Gln Val Leu Leu Leu Arg Thr Arg Pro Val Leu Trp Val Gly Val Ser  
 610 615 620  
 Met Gln Phe Ile Pro Ala Glu Ile Pro Arg Ser Ala Phe Glu Cys Arg  
 625 630 635 640  
 Glu Gln Val Val Ser Glu Gln Thr Leu Val Gln Ser Asn Ile Cys Leu  
 645 650 655  
 Tyr Ile Asp Lys Arg Ser Lys Asn Leu Leu Gly Ser Arg Asp Leu Gln  
 660 665 670

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Ser Ser Val Thr Leu Asp Leu Ala Leu Ala Pro Gly Arg Leu Ser Pro  
 675 680 685  
 Arg Ala Ile Phe Gln Glu Thr Lys Asn Arg Ser Leu Ser Arg Val Arg  
 690 700  
 Val Leu Gly Leu Lys Ala His Cys Glu Asn Phe Asn Leu Leu Leu Pro  
 705 710 715 720  
 Ser Cys Val Glu Asp Ser Val Ile Pro Ile Ile Leu Arg Leu Asn Phe  
 725 730 735  
 Thr Leu Val Gly Lys Pro Leu Leu Ala Phe Arg Asn Leu Arg Pro Met  
 740 745 750  
 Leu Ala Ala Leu Ala Gln Arg Tyr Phe Thr Ala Ser Leu Pro Phe Glu  
 755 760 765  
 Lys Asn Cys Gly Ala Asp His Ile Cys Gln Asp Asn Leu Gly Ile Ser  
 770 775 780  
 Phe Ser Phe Pro Gly Leu Lys Ser Leu Leu Val Gly Ser Asn Leu Glu  
 785 790 795 800  
 Leu Asn Ala Glu Val Met Val Trp Asn Asp Gly Glu Asp Ser Tyr Gly  
 805 810 815  
 Thr Thr Ile Thr Phe Ser His Pro Ala Gly Leu Ser Tyr Arg Tyr Val  
 820 825 830  
 Ala Glu Gly Gln Lys Gln Gly Gln Leu Arg Ser Leu His Leu Thr Cys  
 835 840 845  
 Cys Ser Ala Pro Val Gly Ser Gln Gly Thr Trp Ser Thr Ser Cys Arg  
 850 855 860  
 Ile Asn His Leu Ile Phe Arg Gly Gly Ala Gln Ile Thr Phe Leu Ala  
 865 870 875 880  
 Thr Phe Asp Val Ser Pro Lys Ala Val Gly Leu Asp Arg Leu Leu Leu  
 885 890 895  
 Ile Ala Asn Val Ser Ser Glu Asn Asn Ile Pro Arg Thr Ser Lys Thr  
 900 905 910  
 Ile Phe Gln Leu Glu Leu Pro Val Lys Tyr Ala Val Tyr Ile Val Val  
 915 920 925  
 Ser Ser His Glu Gln Phe Thr Lys Tyr Leu Asn Phe Ser Glu Ser Glu  
 930 935 940  
 Glu Lys Glu Ser His Val Ala Met His Arg Tyr Gln Val Asn Asn Leu  
 945 950 955 960  
 Gly Gln Arg Asp Leu Pro Val Ser Ile Asn Phe Trp Val Pro Val Glu  
 965 970 975  
 Leu Asn Gln Glu Ala Val Trp Met Asp Val Glu Val Ser His Pro Gln  
 980 985 990

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Asn Pro Ser Leu Arg Cys Ser Ser Glu Lys Ile Ala Pro Pro Ala Ser
 995                                1000                    1005

Asp Phe Leu Ala His Ile Gln Lys Asn Pro Val Leu Asp Cys Ser Ile
 1010                                1015                    1020

Ala Gly Cys Leu Arg Phe Arg Cys Asp Val Pro Ser Phe Ser Val Gln
 1025                                1030                    1035                    1040

Glu Glu Leu Asp Phe Thr Leu Lys Gly Asn Leu Ser Phe Gly Trp Val
 1045                                1050                    1055

Arg Gln Ile Leu Gln Lys Lys Val Ser Val Val Ser Val Ala Glu Ile
 1060                                1065                    1070

Ile Phe Asp Thr Ser Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala Phe
 1075                                1080                    1085

Met Arg Ala Gln Thr Ile Thr Val Leu Glu Lys Tyr Lys Val His Asn
 1090                                1095                    1100

Pro Ile Pro Leu Ile Val Gly Ser Ser Ile Gly Gly Leu Leu Leu Leu
 1105                                1110                    1115                    1120

Ala Leu Ile Thr Ala Val Leu Tyr Lys Val Gly Phe Phe Lys Arg Gln
 1125                                1130                    1135

Tyr Lys Glu Met Met Glu Glu Ala Asn Gly Gln Ile Ala Pro Glu Asn
 1140                                1145                    1150

Gly Thr Gln Thr Pro Ser Pro Pro Ser Glu Lys
 1155                                1160

```

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Phe Asn Leu Asp Val Glu Glu Pro Met Val Phe Gln
 1          5          10

```

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:-
- (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTYAAYTGG AYGTINGARGA RCCNATGGTN TTYCA

35

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTCAACCTGG ACGTGGAGGA GCCCATGGTG TTCCAA

36

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTCAACCTGG ACGTNGAASA NCCCATGGTC TTCCAA

36

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTYAAYTNG AYGTINGARGA RCC

23

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTYAAYTGG ACGTNGAAGA

20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGRAANACCA TNGGYTC

17

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTGGAAGACC ATNGGYTC

18

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATTAACCCTC ACTAAAG

17



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## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATACGACTC ACTATAG

17

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 11 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Val	Phe	Gln	Glu	Xaa	Gly	Ala	Gly	Phe	Gly	Gln
1				5					10	

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 14 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Leu	Tyr	Asp	Xaa	Val	Ala	Ala	Thr	Gly	Leu	Xaa	Gln	Pro	Ile
1				5					10				

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro Leu Glu Tyr Xaa Asp Val Ile Pro Gln Ala Glu  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Phe Gln Glu Gly Phe Ser Xaa Val Leu Xaa  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Thr Ser Pro Thr Phe Ile Xaa Met Ser Gln Glu Asn Val Asp  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Val Val Gly Ala Pro Leu Glu Val Val Ala Val Xaa Gln Thr Gly  
1 5 10 15  
Arg

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Leu Asp Xaa Lys Pro Xaa Asp Thr Ala  
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Phe Gly Glu Gln Phe Ser Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

RAANCCYTCY TGRAAACTYT C

21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1006 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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TTCAACCTGG ACGTGGAGGA GCCCATGGTG TTCAAGAGGA TGGAGCTGGC TTTGGACAGA	60
GCGTGGCCCA GCTTGGCGGA TCTAGACTCG TGGTGGGAGC CCCCTGGAG GTGGTGGCGG	120
TCAACCAAAC AGGAAGGTTG TATGACTGTG TGGCTGCCAC TGGCCTTGTC AACCCATACC	180
CCTGCACACA CCCCAGATG CTGTGAACAT GTCCCTGGGT CTGTCCCTGT CAGCCGCCGC	240
CAGTCGCCCC TGGCTGCTGG CCTGTGGCCC AACCATGCAC AGAGCCTGTG GGGAGAATAT	300
GTATGCAGAA GGCTTTTGCC TCCTGTTGGA CTCCCATCTG CAGACCATTG GGACAGTACC	360
TGCTGCCCTA CCAGAGTGTC CAAGTCAAGA GATGGACATT GTCTTCCTGA TTGATGGTTC	420
TGGCAGTATG AGCAAAGTGA CTTTAAACAA ATGAAGGATT TGTGAGAGCT GTGATGGGAC	480
AGTTTGAGGG CACCCAAACC CTGTTCTCAC TGATACAGTA TCCCACCTCC CTGAAGATCC	540
ACTTCACCTT CACGCAATTC CAGAGCAGCT GGAACCCTCT GAGCCTGGTG GATCCCATTG	600
TCCAACTGGA CGGCCTGACA TATACAGCCA CGGGCATCCG GAAAGTGGTG GAGGAACTGT	660
TTCATAGTAA GAATGGGGCC CGTAAAAGTG CCAAGAAGAT CCTCATTGTC ATCACAGATG	720
GCAAAAATAC AAAGACCCCC TGGAGTACGA GGACGTATCC CCAGGCAGAG AGAGCGGATC	780
ATCCGCTATG CCATTGGGGT GGGAGATGCT TTCTGGAAC CCAGTGCCAA GCAGGAGCTG	840
GACAACATTG GCTCAGAGCC GGCTCAGGAC CATGTCTTCA GGGTGGACAA CTTTGCAGCA	900
CTCAGCAGCA TCCAGGAGCA GCTGCAGGAG AAGATCTTTG CACTCGAAGG AACCCAGTCG	960
ACGACAAGTA GCTCTTTCCA ACATGAGATG TTCCAAGAAG GGTTC	1006

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTNTTYCARG ARGAYGG

17

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCACTGTCAG GATGCCCCGTG

20

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGTTACGAAT TCGCCACCAT GGCTCTACGG GTGCTTCTTC TG

42

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGTTACGAAT TCGCCACCAT GACTCGGACT GTGCTTCTTC TG

42

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGTTACGAAT TCGCCACCAT GACCTTCGGC ACTGTG

36

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTGCTGACTG CCTGCAGTTC

20

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTTCTGACGC GTAATGGCAT TCTAGACCTC GTCTTC

36

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACGTATGCAG GATCCCATCA AGAGATGGAC ATCGCT

36

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 37 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACTGCATGTC TCGAGGCTGA AGCCTTCTTG GGACATC

37

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TATAGACTGC TGGGTAGTCC CCAC

24

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGAAGATTGC CGCTAAATAA CAGA

24

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 3528 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..3456

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGC TGG GCC CTG GCT TCC TGT CAT GGG TCT AAC CTG GAT GTG GAG GAA	48
Gly Trp Ala Leu Ala Ser Cys His Gly Ser Asn Leu Asp Val Glu Glu	
1 5 10 15	
CCC ATC GTG TTC AGA GAG GAT GCA GCC AGC TTT GGA CAG ACT GTG GTG	96
Pro Ile Val Phe Arg Glu Asp Ala Ala Ser Phe Gly Gln Thr Val Val	
20 25 30	
CAG TTT GGT GGA TCT CGA CTC GTG GTG GGA GCC CCT CTG GAG GCG GTG	144
Gln Phe Gly Gly Ser Arg Leu Val Val Gly Ala Pro Leu Glu Ala Val	
35 40 45	
GCA GTC AAC CAA ACA GGA CGG TTG TAT GAC TGT GCA CCT GCC ACT GGC	192
Ala Val Asn Gln Thr Gly Arg Leu Tyr Asp Cys Ala Pro Ala Thr Gly	
50 55 60	

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ATG TGC CAG CCC ATC GTA CTG CGC AGT CCC CTA GAG GCA GTG AAC ATG Met Cys Gln Pro Ile Val Leu Arg Ser Pro Leu Glu Ala Val Asn Met 65 70 75 80	240
TCC CTG GGC CTG TCT CTG GTG ACT GCC ACC AAT AAC GCC CAG TTG CTG Ser Leu Gly Leu Ser Leu Val Thr Ala Thr Asn Asn Ala Gln Leu Leu 85 90 95	288
GCT TGT GGT CCA ACT GCA CAG AGA GCT TGT GTG AAG AAC ATG TAT GCG Ala Cys Gly Pro Thr Ala Gln Arg Ala Cys Val Lys Asn Met Tyr Ala 100 105 110	336
AAA GGT TCC TGC CTC CTT CTC GGC TCC AGC TTG CAG TTC ATC CAG GCA Lys Gly Ser Cys Leu Leu Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala 115 120 125	384
GTC CCT GCC TCC ATG CCA GAG TGT CCA AGA CAA GAG ATG GAC ATT GCT Val Pro Ala Ser Met Pro Glu Cys Pro Arg Gln Glu Met Asp Ile Ala 130 135 140	432
TTT CTG ATT GAT GGT TCT GGC AGC ATT AAC CAA AGG GAC TTT GCC CAG Phe Leu Ile Asp Gly Ser Gly Ser Ile Asn Gln Arg Asp Phe Ala Gln 145 150 155 160	480
ATG AAG GAC TTT GTC AAA GCT TTG ATG GGA GAG TTT GCG AGC ACC AGC Met Lys Asp Phe Val Lys Ala Leu Met Gly Glu Phe Ala Ser Thr Ser 165 170 175	528
ACC TTG TTC TCC CTG ATG CAA TAC TCG AAC ATC CTG AAG ACC CAT TTT Thr Leu Phe Ser Leu Met Gln Tyr Ser Asn Ile Leu Lys Thr His Phe 180 185 190	576
ACC TTC ACT GAA TTC AAG AAC ATC CTG GAC CCT CAG AGC CTG GTG GAT Thr Phe Thr Glu Phe Lys Asn Ile Leu Asp Pro Gln Ser Leu Val Asp 195 200 205	624
CCC ATT GTC CAG CTG CAA GGC CTG ACC TAC ACA GCC ACA GGC ATC CGG Pro Ile Val Gln Leu Gln Gly Leu Thr Tyr Thr Ala Thr Gly Ile Arg 210 215 220	672
ACA GTG ATG GAA GAG CTA TTT CAT AGC AAG AAT GGG TCC CGT AAA AGT Thr Val Met Glu Glu Leu Phe His Ser Lys Asn Gly Ser Arg Lys Ser 225 230 235 240	720
GCC AAG AAG ATC CTC CTT GTC ATC ACA GAT GGG CAG AAA TAC AGA GAC Ala Lys Lys Ile Leu Leu Val Ile Thr Asp Gly Gln Lys Tyr Arg Asp 245 250 255	768
CCC CTG GAG TAT AGT GAT GTC ATT CCC GCC GCA GAC AAA GCT GGC ATC Pro Leu Glu Tyr Ser Asp Val Ile Pro Ala Ala Asp Lys Ala Gly Ile 260 265 270	816
ATT CGT TAT GCT ATT GGG GTG GGA GAT GCC TTC CAG GAG CCC ACT GCC Ile Arg Tyr Ala Ile Gly Val Gly Asp Ala Phe Gln Glu Pro Thr Ala 275 280 285	864
CTG AAG GAG CTG AAC ACC ATT GGC TCA GCT CCC CCA CAG GAC CAC GTG Leu Lys Glu Leu Asn Thr Ile Gly Ser Ala Pro Pro Gln Asp His Val 290 295 300	912



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TTC AAG GTA GGC AAC TTT GCA GCA CTT CGC AGC ATC CAG AGG CAA CTT Phe Lys Val Gly Asn Phe Ala Ala Leu Arg Ser Ile Gln Arg Gln Leu 305 310 315 320	960
CAG GAG AAA ATC TTC GCC ATT GAG GGA ACT CAA TCA AGG TCA AGT AGT Gln Glu Lys Ile Phe Ala Ile Glu Gly Thr Gln Ser Arg Ser Ser Ser 325 330 335	1008
TCC TTT CAG CAC GAG ATG TCA CAA GAA GGT TTC AGT TCA GCT CTC ACA Ser Phe Gln His Glu Met Ser Gln Glu Gly Phe Ser Ser Ala Leu Thr 340 345 350	1056
TCG GAT GGA CCC GTT CTG GGG GCC GYG GGA AGC TTC AGC TGG TCC GGA Ser Asp Gly Pro Val Leu Gly Ala Xaa Gly Ser Phe Ser Trp Ser Gly 355 360 365	1104
GGT GCC TTC TTA TAT CCC CCA AAT ACG AGA CCC ACC TTT ATC AAC ATG Gly Ala Phe Leu Tyr Pro Pro Asn Thr Arg Pro Thr Phe Ile Asn Met 370 375 380	1152
TCT CAG GAG AAT GTG GAC ATG AGA GAC TCC TAC CTG GGT TAC TCC ACC Ser Gln Glu Asn Val Asp Met Arg Asp Ser Tyr Leu Gly Tyr Ser Thr 385 390 395 400	1200
GCA GTG GCC TTT TGG AAG GGG GTT CAC AGC CTG ATC CTG GGG GCC CCG Ala Val Ala Phe Trp Lys Gly Val His Ser Leu Ile Leu Gly Ala Pro 405 410 415	1248
CGT CAC CAG CAC ACG GGG AAG GTT GTC ATC TTT ACC CAG GAA GCC AGG Arg His Gln His Thr Gly Lys Val Ile Phe Thr Gln Glu Ala Arg 420 425 430	1296
CAT TGG AGG CCC AAG TCT GAA GTC AGA GGG ACA CAG ATC GGC TCC TAC His Trp Arg Pro Lys Ser Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr 435 440 445	1344
TTC GGG GCC TCT CTC TGT TCT GTG GAC CTG GAT AGA GAT GGC AGC ACY Phe Gly Ala Ser Leu Cys Ser Val Asp Val Asp Arg Asp Gly Ser Xaa 450 455 460	1392
GAC CTG GTC CTG ATC GGA GCC CCC CAT TAC TAT GAG CAG ACC CGA GGG Asp Leu Val Leu Ile Gly Ala Pro His Tyr Tyr Glu Gln Thr Arg Gly 465 470 475 480	1440
GGG CAG GTC TCA GTG TKC CCC GTG CCC GGT GTG AGG GGC AGG TGG CAG Gly Gln Val Ser Val Xaa Pro Val Pro Gly Val Arg Gly Arg Trp Gln 485 490 495	1488
TGT GAG GCC ACC CTC CAC GGG GAG CAG GRC CAT CCT TGG GGC CGC TTT Cys Glu Ala Thr Leu His Gly Glu Gln Xaa His Pro Trp Gly Arg Phe 500 505 510	1536
GGG GTG GCT CTG ACA GTG CTG GGG GAC GTA AAC GGG GAC AAT CTG GCA Gly Val Ala Leu Thr Val Leu Gly Asp Val Asn Gly Asp Asn Leu Ala 515 520 525	1584
GAC GTG GCT ATT GGT GCC CCT GGA GAG GAG GAG AGC AGA GGT GCT GTC Asp Val Ala Ile Gly Ala Pro Gly Glu Glu Glu Ser Arg Gly Ala Val 530 535 540	1632

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TAC ATA TTT CAT GGA GCC TCG AGA CTG GAG ATC ATG CCC TCA CCC AGC Tyr Ile Phe His Gly Ala Ser Arg Leu Glu Ile Met Pro Ser Pro Ser 545 550 555 560	1680
CAG CGG GTC ACT GGC TCC CAG CTC TCC CTG AGA CTG CAG TAT TTT GGG Gln Arg Val Thr Gly Ser Gln Leu Ser Leu Arg Leu Gln Tyr Phe Gly 565 570 575	1728
CAG TCA TTG AGT GGG GGT CAG GAC CTT ACA CAG GAT GGC CTG GTG GAC Gln Ser Leu Ser Gly Gly Gln Asp Leu Thr Gln Asp Gly Leu Val Asp 580 585 590	1776
CTG GCC GTG GGA GCC CAG GGG CAC GTA CTG CTG CTC AGG AGT CTG CCT Leu Ala Val Gly Ala Gln Gly His Val Leu Leu Leu Arg Ser Leu Pro 595 600 605	1824
CTG CTG AAA GTG GAG CTC TCC ATA AGA TTC GCC CCC ATG GAG GTG GCA Leu Leu Lys Val Glu Leu Ser Ile Arg Phe Ala Pro Met Glu Val Ala 610 615 620	1872
AAG GCT GTG TAC CAG TGC TCG GAA AGG ACT CCC ACT GTC CTC GAA GCT Lys Ala Val Tyr Gln Cys Trp Glu Arg Thr Pro Thr Val Leu Glu Ala 625 630 635 640	1920
GGA GAG GCC ACT GTC TGT CTC ACT GTC CAC AAA GGC TCA CCT GAC CTG Gly Glu Ala Thr Val Cys Leu Thr Val His Lys Gly Ser Pro Asp Leu 645 650 655	1968
TTA GGT AAT GTC CAA GCC TCT GTC AGG TAT GAT CTG GCG TTA GAT CCG Leu Gly Asn Val Gln Gly Ser Val Tyr Asp Leu Ala Leu Asp Pro 660 665 670	2016
GGC CGC CTG ATT TCT CGT GCC ATT TTT GAT GAG ACT AAG AAC TGC ACT Gly Arg Leu Ile Ser Arg Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr 675 680 685	2064
TTG ACG GGA AGG AAG ACT CTG GGC CTT GGT GAT CAC TGC GAA ACA GTG Leu Thr Gly Arg Lys Thr Leu Gly Leu Gly Asp His Cys Glu Thr Val 690 695 700	2112
AAG CTG CTT TTG CCG GAC TGT GTG GAA GAT GCA GTG AGC CCT ATC ATC Lys Leu Leu Leu Pro Asp Cys Val Glu Asp Ala Val Ser Pro Ile Ile 705 710 715 720	2160
CTG CGC CTC AAC TTT TCC CTG GTG AGA GAC TCT GCT TCA CCC AGG AAC Leu Arg Leu Asn Phe Ser Leu Val Arg Asp Ser Ala Ser Pro Arg Asn 725 730 735	2208
CTG CAT CCT GTG CTG GCT GTG GGC TCA CAA GAC CAC ATA ACT GCT TCT Leu His Pro Val Leu Ala Val Gly Ser Gln Asp His Ile Thr Ala Ser 740 745 750	2256
CTG CCG TTT GAG AAG AAC TGT AAG CAA GAA CTC CTG TGT GAG GGC GAC Leu Pro Phe Glu Lys Asn Cys Lys Gln Glu Leu Leu Cys Glu Gly Asp 755 760 765	2304
CTG GGC ATC AGC TTT AAC TTC TCA GGC CTG CAG GTC TTG GTG GTG GGA Leu Gly Ile Ser Phe Asn Phe Ser Gly Leu Gln Val Leu Val Val Gly 770 775 780	2352

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GGC TCC CCA CAG CTC ACT GTG ACA GTC ACT GTG TGG AAT GAG GGT GAG Gly Ser Pro Glu Leu Thr Val Thr Val Thr Val Trp Asn Glu Gly Glu 785 790 795 800	2400
GAC AGC TAT GGA ACT TTA GTC AAG TTC TAC TAC CCA GCA GGG CTA TCT Asp Ser Tyr Gly Thr Leu Val Lys Phe Tyr Tyr Pro Ala Gly Leu Ser 805 810 815	2448
TAC CGA CGG GTA ACA GGG ACT CAG CAA CCT CAT CAG TAC CCA CTA CGC Tyr Arg Arg Val Thr Gly Thr Gln Gln Pro His Gln Tyr Pro Leu Arg 820 825 830	2496
TTG GCC TGT GAG GCT GAG CCC GCT GCC CAG GAG GAC CTG AGG AGC AGC Leu Ala Cys Glu Ala Glu Pro Ala Ala Gln Glu Asp Leu Arg Ser Ser 835 840 845	2544
AGC TGT AGC ATT AAT CAC CCC ATC TTC CGA GAA GGT GCA AAG ACC ACC Ser Cys Ser Ile Asn His Pro Ile Phe Arg Glu Gly Ala Lys Thr Thr 850 855 860	2592
TTC ATG ATC ACA TTC GAT GTC TCC TAC AAG GCC TTC CTA GGA GAC AGG Phe Met Ile Thr Phe Asp Val Ser Tyr Lys Ala Phe Leu Gly Asp Arg 865 870 875 880	2640
TTG CTT CTG AGG GCC AAA GCC AGC AGT GAG AAT AAT AAG CCT GAT ACC Leu Leu Leu Arg Ala Lys Ala Ser Ser Glu Asn Asn Lys Pro Asp Thr 885 890 895	2688
AAC AAG ACT GCC TTC CAG CTG GAG CTC CCA GTG AAG TAC ACC GTC TAT Asn Lys Thr Ala Phe Gln Leu Glu Leu Pro Val Lys Tyr Thr Val Tyr 900 905 910	2736
ACC CTG ATC ACT AGG CAA GAA GAT TCC ACC AAC CAT GTC AAC TTT TCA Thr Leu Ile Ser Arg Gln Glu Asp Ser Thr Asn His Val Asn Phe Ser 915 920 925	2784
TCT TCC CAC GGG GGG AGA AGG CAA GAA GCC GCA CAT CGC TAT CGT GTG Ser Ser His Gly Gly Arg Arg Gln Glu Ala Ala His Arg Tyr Arg Val 930 935 940	2832
AAT AAC CTG AGT CCA CTG AAG CTG GCC GTC AGA GTT AAC TTC TGG GTC Asn Asn Leu Ser Pro Leu Lys Leu Ala Val Arg Val Asn Phe Trp Val 945 950 955 960	2880
CCT GTC CTT CTG AAC CGT GTG GCT GTG TGG GAC GTG ACT CTG AGC AGC Pro Val Leu Leu Asn Gly Val Ala Val Trp Asp Val Thr Leu Ser Ser 965 970 975	2928
CCA GCA CAG GGT GTC TCC TGC GTG TCC CAG ATG AAA CCT CCT CAG AAT Pro Ala Gln Gly Val Ser Cys Val Ser Gln Met Lys Pro Pro Gln Asn 980 985 990	2976
CCC GAC TTT CTG ACC CAG ATT CAG AGA CGT TCT GTG CTG GAC TGC TCC Pro Asp Phe Leu Thr Gln Ile Gln Arg Arg Ser Val Leu Asp Cys Ser 995 1000 1005	3024
ATT GCT GAC TGC CTG CAC TCC CGC TGT GAC ATC CCC TCC TTG GAC ATC Ile Ala Asp Cys Leu His Ser Arg Cys Asp Ile Pro Ser Leu Asp Ile 1010 1015 1020	3072

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CAC GAT GAA CTT GAC TTC ATT CTG AGG GGC AAC CTC AGC TTC GGC TGG Gln Asp Glu Leu Asp Phe Ile Leu Arg Gly Asn Leu Ser Phe Gly Trp 1025 1030 1035 1040	3120
GTC AGT CAG ACA TTG CAG GAA AAG GTG TTG CTT GTG AGT GAG GCT GAA Val Ser Gln Thr Leu Gln Glu Lys Val Leu Leu Val Ser Glu Ala Glu 1045 1050 1055	3168
ATC ACT TTC GAC ACA TCT GTG TAC TCC CAG CTG CCA GGA CAG GAG GCA Ile Thr Phe Asp Thr Ser Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala 1060 1065 1070	3216
TTT CTG AGA GCC CAG CTG GAG ACA ACG TTA GAA GAA TAC GTG GTC TAT Phe Leu Arg Ala Gln Val Glu Thr Thr Leu Glu Glu Tyr Val Val Tyr 1075 1080 1085	3264
GAG CCC ATC TTC CTC GTG GCG GGC AGC TCG GTG GGA GGT CTG CTG TTA Glu Pro Ile Phe Leu Val Ala Gly Ser Ser Val Gly Gly Leu Leu Leu 1090 1095 1100	3312
CTG GCT CTC ATC ACA GTG GTA CTG TAC AAG CTT GGC TYC TYC AAA CGT Leu Ala Leu Ile Thr Val Val Leu Tyr Lys Leu Gly Xaa Xaa Lys Arg 1105 1110 1115 1120	3360
CAG TAC AAA GAA ATG CTG GAC GGC AAG GCT GCA GAT CCT GTC ACA GCC Gln Tyr Lys Glu Met Leu Asp Gly Lys Ala Ala Asp Pro Val Thr Ala 1125 1130 1135	3408
GGC CAG GCA GAT TTC GGC TGT GAG ACT CCT CCA TAT CTC GTG AGC TAGGAATCCA Gly Gln Ala Asp Phe Gly Cys Glu Thr Pro Pro Tyr Leu Val Ser 1140 1145 1150	3463
CTCTCCTGCC TATCTCTGNA ATGAAGATTG GTCCTGCCTA TGAGTCTACT GGCATGGGAA	3523
CGAGT	3528

## (2) INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Gly Trp Ala Leu Ala Ser Cys His Gly Ser Asn Leu Asp Val Glu Glu 1 5 10 15
Pro Ile Val Phe Arg Glu Asp Ala Ala Ser Phe Gly Gln Thr Val Val 20 25 30
Gln Phe Gly Gly Ser Arg Leu Val Val Gly Ala Pro Leu Glu Ala Val 35 40 45
Ala Val Asn Gln Thr Gly Arg Leu Tyr Asp Cys Ala Pro Ala Thr Gly 50 55 60

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Met Cys Gln Pro Ile Val Leu Arg Ser Pro Leu Glu Ala Val Asn Met  
 65 70 75 80  
 Ser Leu Gly Leu Ser Leu Val Thr Ala Thr Asn Asn Ala Gln Leu Leu  
 85 90 95  
 Ala Cys Gly Pro Thr Ala Gln Arg Ala Cys Val Lys Asn Met Tyr Ala  
 100 105 110  
 Lys Gly Ser Cys Leu Leu Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala  
 115 120 125  
 Val Pro Ala Ser Met Pro Glu Cys Pro Arg Gln Glu Met Asp Ile Ala  
 130 135 140  
 Phe Leu Ile Asp Gly Ser Gly Ser Ile Asn Gln Arg Asp Phe Ala Gln  
 145 150 155 160  
 Met Lys Asp Phe Val Lys Ala Leu Met Gly Glu Phe Ala Ser Thr Ser  
 165 170 175  
 Thr Leu Phe Ser Leu Met Gln Tyr Ser Asn Ile Leu Lys Thr His Phe  
 180 185 190  
 Thr Phe Thr Glu Phe Lys Asn Ile Leu Asp Pro Gln Ser Leu Val Asp  
 195 200 205  
 Pro Ile Val Gln Leu Gln Gly Leu Thr Tyr Thr Ala Thr Gly Ile Arg  
 210 215 220  
 Thr Val Met Glu Glu Leu Phe His Ser Lys Asn Gly Ser Arg Lys Ser  
 225 230 235 240  
 Ala Lys Lys Ile Leu Leu Val Ile Thr Asp Gly Gln Lys Tyr Arg Asp  
 245 250 255  
 Pro Leu Glu Tyr Ser Asp Val Ile Pro Ala Ala Asp Lys Ala Gly Ile  
 260 265 270  
 Ile Arg Tyr Ala Ile Gly Val Gly Asp Ala Phe Gln Glu Pro Thr Ala  
 275 280 285  
 Leu Lys Glu Leu Asn Thr Ile Gly Ser Ala Pro Pro Gln Asp His Val  
 290 295 300  
 Phe Lys Val Gly Asn Phe Ala Ala Leu Arg Ser Ile Gln Arg Gln Leu  
 305 310 315 320  
 Gln Glu Lys Ile Phe Ala Ile Glu Gly Thr Gln Ser Arg Ser Ser Ser  
 325 330 335  
 Ser Phe Gln His Glu Met Ser Gln Glu Gly Phe Ser Ser Ala Leu Thr  
 340 345 350  
 Ser Asp Gly Pro Val Leu Gly Ala Xaa Gly Ser Phe Ser Trp Ser Gly  
 355 360 365  
 Gly Ala Phe Leu Tyr Pro Pro Asn Thr Arg Pro Thr Phe Ile Asn Met  
 370 375 380

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Ser Gln Glu Asn Val Asp Met Arg Asp Ser Tyr Leu Gly Tyr Ser Thr  
 385 390 395 400  
 Ala Val Ala Phe Trp Lys Gly Val His Ser Leu Ile Leu Gly Ala Pro  
 405 410 415  
 Arg His Gln His Thr Gly Lys Val Val Ile Phe Thr Gln Glu Ala Arg  
 420 425 430  
 His Trp Arg Pro Lys Ser Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr  
 435 440 445  
 Phe Gly Ala Ser Leu Cys Ser Val Asp Val Asp Arg Asp Gly Ser Xaa  
 450 455 460  
 Asp Leu Val Leu Ile Gly Ala Pro His Tyr Tyr Glu Gln Thr Arg Gly  
 465 470 475 480  
 Gly Gln Val Ser Val Xaa Pro Val Pro Gly Val Arg Gly Arg Trp Gln  
 485 490 495  
 Cys Glu Ala Thr Leu His Gly Glu Gln Xaa His Pro Trp Gly Arg Phe  
 500 505 510  
 Gly Val Ala Leu Thr Val Leu Gly Asp Val Asn Gly Asp Asn Leu Ala  
 515 520 525  
 Asp Val Ala Ile Gly Ala Pro Gly Glu Glu Glu Ser Arg Gly Ala Val  
 530 535 540  
 Tyr Ile Phe His Gly Ala Ser Arg Leu Glu Ile Met Pro Ser Pro Ser  
 545 550 555 560  
 Gln Arg Val Thr Gly Ser Gln Leu Ser Leu Arg Leu Gln Tyr Phe Gly  
 565 570 575  
 Gln Ser Leu Ser Gly Gly Gln Asp Leu Thr Gln Asp Gly Leu Val Asp  
 580 585 590  
 Leu Ala Val Gly Ala Gln Gly His Val Leu Leu Leu Arg Ser Leu Pro  
 595 600 605  
 Leu Leu Lys Val Glu Leu Ser Ile Arg Phe Ala Pro Met Glu Val Ala  
 610 615 620  
 Lys Ala Val Tyr Gln Cys Trp Glu Arg Thr Pro Thr Val Leu Glu Ala  
 625 630 635 640  
 Gly Glu Ala Thr Val Cys Leu Thr Val His Lys Gly Ser Pro Asp Leu  
 645 650 655  
 Leu Gly Asn Val Gln Gly Ser Val Arg Tyr Asp Leu Ala Leu Asp Pro  
 660 665 670  
 Gly Arg Leu Ile Ser Arg Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr  
 675 680 685  
 Leu Thr Gly Arg Lys Thr Leu Gly Leu Gly Asp His Cys Glu Thr Val  
 690 695 700

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Lys Leu Leu Leu Pro Asp Cys Val Glu Asp Ala Val Ser Pro Ile Ile  
 705 710 715 720  
 Leu Arg Leu Asn Phe Ser Leu Val Arg Asp Ser Ala Ser Pro Arg Asn  
 725 730 735  
 Leu His Pro Val Leu Ala Val Gly Ser Gln Asp His Ile Thr Ala Ser  
 740 745 750  
 Leu Pro Phe Glu Lys Asn Cys Lys Gln Glu Leu Leu Cys Glu Gly Asp  
 755 760 765  
 Leu Gly Ile Ser Phe Asn Phe Ser Gly Leu Gln Val Leu Val Val Gly  
 770 775 780  
 Gly Ser Pro Glu Leu Thr Val Thr Val Thr Val Trp Asn Glu Gly Glu  
 785 790 795 800  
 Asp Ser Tyr Gly Thr Leu Val Lys Phe Tyr Tyr Pro Ala Gly Leu Ser  
 805 810 815  
 Tyr Arg Arg Val Thr Gly Thr Gln Gln Pro His Gln Tyr Pro Leu Arg  
 820 825 830  
 Leu Ala Cys Glu Ala Glu Pro Ala Ala Gln Glu Asp Leu Arg Ser Ser  
 835 840 845  
 Ser Cys Ser Ile Asn His Pro Ile Phe Arg Glu Gly Ala Lys Thr Thr  
 850 855 860  
 Phe Met Ile Thr Phe Asp Val Ser Tyr Lys Ala Phe Leu Gly Asp Arg  
 865 870 875 880  
 Leu Leu Leu Arg Ala Lys Ala Ser Ser Glu Asn Asn Lys Pro Asp Thr  
 885 890 895  
 Asn Lys Thr Ala Phe Gln Leu Glu Leu Pro Val Lys Tyr Thr Val Tyr  
 900 905 910  
 Thr Leu Ile Ser Arg Gln Glu Asp Ser Thr Asn His Val Asn Phe Ser  
 915 920 925  
 Ser Ser His Gly Gly Arg Arg Gln Glu Ala Ala His Arg Tyr Arg Val  
 930 935 940  
 Asn Asn Leu Ser Pro Leu Lys Leu Ala Val Arg Val Asn Phe Trp Val  
 945 950 955 960  
 Pro Val Leu Leu Asn Gly Val Ala Val Trp Asp Val Thr Leu Ser Ser  
 965 970 975  
 Pro Ala Gln Gly Val Ser Cys Val Ser Gln Met Lys Pro Pro Gln Asn  
 980 985 990  
 Pro Asp Phe Leu Thr Gln Ile Gln Arg Arg Ser Val Leu Asp Cys Ser  
 995 1000 1005  
 Ile Ala Asp Cys Leu His Ser Arg Cys Asp Ile Pro Ser Leu Asp Ile  
 1010 1015 1020

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Gln Asp Glu Leu Asp Phe Ile Leu Arg Gly Asn Leu Ser Phe Gly Trp  
 1025 1030 1035 1040

Val Ser Gln Thr Leu Gln Glu Lys Val Leu Leu Val Ser Glu Ala Glu  
 1045 1050 1055

Ile Thr Phe Asp Thr Ser Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala  
 1060 1065 1070

Phe Leu Arg Ala Gln Val Glu Thr Thr Leu Glu Glu Tyr Val Val Tyr  
 1075 1080 1085

Glu Pro Ile Phe Leu Val Ala Gly Ser Ser Val Gly Gly Leu Leu Leu  
 1090 1095 1100

Leu Ala Leu Ile Thr Val Val Leu Tyr Lys Leu Gly Xaa Xaa Lys Arg  
 1105 1110 1115 1120

Gln Tyr Lys Glu Met Leu Asp Gly Lys Ala Ala Asp Pro Val Thr Ala  
 1125 1130 1135

Gly Gln Ala Asp Phe Gly Cys Glu Thr Pro Pro Tyr Leu Val Ser  
 1140 1145 1150

## (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GTCCAAGCTG TCATGGGCCA G

21

## (2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTCCAGCAGA CTGAAGAGCA CGG

23

## (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid



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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

18

TGTAACACGA CGGCCAGT

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

19

GGAAACAGCT ATGACCATG

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

22

GGACATGTTC ACTGCCTCTA GG

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

25

GGCGGACAGT CAGACGACTG TCCTG

(2) INFORMATION FOR SEQ ID NO:44:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 38 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTGGTTCGGC CCACCTCTGA AGGTTCCAGA ATCGATAG

38

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 3519 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 52..3519

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCTTTCTGAA GGTTCAGAA TCCATAGTA ATTCGTGGGC ACTGCTCAGA T ATG GTC	57
Met Val	
1	
CGT GGA GTT GTG ATC CTC CTG TGT GGC TGG GCC CTG GCT TCC TGT CAT	105
Arg Gly Val Val Ile Leu Leu Cys Gly Trp Ala Leu Ala Ser Cys His	
5 10 15	
GGG TCT AAC CTG GAT GTG GAG AAG CCC GTC GTG TTC AAA GAG GAT GCA	153
Gly Ser Asn Leu Asp Val Glu Lys Pro Val Val Phe Lys Glu Asp Ala	
20 25 30	
GCC AGC TTC GGA CAG ACT GTG GTG CAG TTT GGT GGA TCT CGA CTC GTG	201
Ala Ser Phe Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg Leu Val	
35 40 45 50	
GTG GGA GCC CCT CTG GAG GCG GTG GCA GTC AAC CAA ACA GGA CAG TCG	249
Val Gly Ala Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly Gln Ser	
55 60 65	
TCT GAC TGT CCG CCT GCC ACT GGC GTG TGC CAG CCC ATC TTA CTG CAC	297
Ser Asp Cys Pro Pro Ala Thr Gly Val Cys Gln Pro Ile Leu Leu His	
70 75 80	
ATT CCC CTA GAG GCA GTG AAC ATG TCC CTG GGC CTG TCT CTG GTG GCT	345
Ile Pro Leu Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu Val Ala	
85 90 95	

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GAC ACC AAT AAC TCC CAG TTG CTG GCT TGT GGT CCA ACT GCA CAG AGA Asp Thr Asn Asn Ser Gln Leu Ala Cys Gly Pro Thr Ala Gln Arg 100 105 110	393
GCT TGT GCA AAG AAC ATG TAT GCA AAA GGT TCC TGC CTC CTT CTG GGC Ala Cys Ala Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu Leu Gly 115 120 125 130	441
TCC AGC TTG CAG TTC ATC CAG GCA ATC CCT GCT ACC ATG CCA GAG TGT Ser Ser Leu Gln Phe Ile Gln Ala Ile Pro Ala Thr Met Pro Glu Cys 135 140 145	489
CCA GGA CAA GAG ATG GAC ATT GCT TTC CTG ATT GAT GGC TCC GGC AGC Pro Gly Gln Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser Gly Ser 150 155 160	537
ATT GAT CAA ACT CAC TTT ACC CAG ATG AAG GAC TTC GTC AAA GCT TTG Ile Asp Gln Ser Asp Phe Thr Gln Met Lys Asp Phe Val Lys Ala Leu 165 170 175	585
ATG GGC CAG TTG GCG AGC ACC AGC ACC TCG TTC TCC CTG ATG CAA TAC Met Gly Gln Leu Ala Ser Thr Ser Thr Ser Phe Ser Leu Met Gln Tyr 180 185 190	633
TCA AAC ATC CTG AAG ACT CAT TTT ACC TTC ACG GAA TTC AAG AGC AGC Ser Asn Ile Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys Ser Ser 195 200 205 210	681
CTG AGC CCT CAG AGC CTG GTG GAT GCC ATC GTC CAG CTC CAA GGC CTG Leu Ser Pro Gln Ser Leu Val Asp Ala Ile Val Gln Leu Gln Gly Leu 215 220 225	729
ACG TAC ACA GCC TCG GGC ATC CAG AAA GTG GTG AAA GAG CTA TTT CAT Thr Tyr Thr Ala Ser Gly Ile Gln Lys Val Val Lys Glu Leu Phe His 230 235 240	777
AGC AAG AAT GGG GCC CGA AAA AGT GCC AAG AAG ATA CTA ATT GTC ATC Ser Lys Asn Gly Ala Arg Lys Ser Ala Lys Lys Ile Leu Ile Val Ile 245 250 255	825
ACA GAT GGG CAG AAA TTC AGA GAC CCC CTG GAG TAT AGA CAT GTC ATC Thr Asp Gly Gln Lys Phe Arg Asp Pro Leu Glu Tyr Arg His Val Ile 260 265 270	873
CCT GAA GCA GAG AAA GCT GGG ATC ATT CGC TAT GCT ATA GGG GTG GGA Pro Glu Ala Glu Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly Val Gly 275 280 285 290	921
GAT GCC TTC CGG GAA CCC ACT GCC CTA CAG GAG CTG AAC ACC ATT GGC Asp Ala Phe Arg Glu Pro Thr Ala Leu Gln Glu Leu Asn Thr Ile Gly 295 300 305	969
TCA GCT CCC TCG CAG GAC CAC GTG TTC AAG GTG GGC AAT TTT GTA GCA Ser Ala Pro Ser Gln Asp His Val Phe Lys Val Gly Asn Phe Val Ala 310 315 320	1017
CTT CGC AGC ATC CAG CGG CAA ATT CAG GAG AAA ATC TTT GCC ATT GAA Leu Arg Ser Ile Gln Arg Gln Ile Gln Glu Lys Ile Phe Ala Ile Glu 325 330 335	1065

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GGA ACC GAA TCA AGG TCA AGT AGT TCC TTT CAG CAC GAG ATG TCA CAA Gly Thr Glu Ser Arg Ser Ser Ser Ser Phe Gln His Glu Met Ser Gln 340 345 350	1113
GAA GGT TTC AGC TCA GCT CTC TCA ATG GAT GGA CCA GTT CTG GGG GCT Glu Gly Phe Ser Ser Ala Leu Ser Met Asp Gly Pro Val Leu Gly Ala 355 360 365 370	1161
GTG GGA GGC TTC AGC TGG TCT GGA GGT GCC TTC TTG TAC CCC TCA AAT Val Gly Gly Phe Ser Trp Ser Gly Gly Ala Phe Leu Tyr Pro Ser Asn 375 380 385	1209
ATG AGA TCC ACC TTC ATC AAC ATG TCT CAG GAG AAC GAG GAT ATG AGG Met Arg Ser Thr Phe Ile Asn Met Ser Gln Glu Asn Glu Asp Met Arg 390 395 400	1257
GAC GCT TAC CTC GGT TAC TCC ACC GCA CTG GCC TTT TGG AAG GGG GTC Asp Ala Tyr Leu Gly Tyr Ser Thr Ala Leu Ala Phe Trp Lys Gly Val 405 410 415	1305
CAC AGC CTG ATC CTG GGG GCC CCT CGC CAC CAG CAC ACG GGG AAG GTT His Ser Leu Ile Leu Gly Ala Pro Arg His Gln His Thr Gly Lys Val 420 425 430	1353
GTC ATC TTT ACC CAG GAA TCC AGG CAC TGG AGG CCC AAG TCT GAA GTC Val Ile Phe Thr Gln Glu Ser Arg His Trp Arg Pro Lys Ser Glu Val 435 440 445 450	1401
AGA GGG ACA CAG ATC GGC TCC TAC TTT GGG GCA TCT CTC TGT TCT GTG Arg Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys Ser Val 455 460 465	1449
GAC ATG GAT AGA GAT GGC AGC ACT GAC CTG GTC CTG ATT GGA GTC CCC Asp Met Asp Arg Asp Gly Ser Thr Asp Leu Val Leu Ile Gly Val Pro 470 475 480	1497
CAT TAC TAT GAG CAC ACC CGA GGG GGG CAG GTG TCG GTG TGC CCC ATG His Tyr Tyr Glu His Thr Arg Gly Gly Gln Val Ser Val Cys Pro Met 485 490 495	1545
CCT GGT GTG AGG AGC AGG TGG CAT TGT GGG ACC ACC CTC CAT GGG GAG Pro Gly Val Arg Ser Arg Trp His Cys Gly Thr Thr Leu His Gly Glu 500 505 510	1593
CAG GGC CAT CCT TGG GGC CGC TTT GGG GCG GCT CTG ACA GTG CTA GGG Gln Gly His Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val Leu Gly 515 520 525 530	1641
GAC GTG AAT GGG GAC AGT CTG GCG GAT GTG GCT ATT GGT GCA CCC GGA Asp Val Asn Gly Asp Ser Leu Ala Asp Val Ala Ile Gly Ala Pro Gly 535 540 545	1689
GAG GAG GAG AAC AGA GGT GCT GTC TAC ATA TTT CAT GGA GCC TCG AGA Glu Glu Glu Asn Arg Gly Ala Val Tyr Ile Phe His Gly Ala Ser Arg 550 555 560	1737
CAG GAC ATC GCT CCC TCG CCT AGC CAG CGG GTC ACT GGC TCC CAG CTC Gln Asp Ile Ala Pro Ser Pro Ser Gln Arg Val Thr Gly Ser Gln Leu 565 570 575	1785

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TTC CTG AGG CTC CAA TAT TTT GGG CAG TCA TTA AGT GGG GGT CAG GAC Phe Leu Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly Gln Asp 580 585 590	1833
CTT ACA CAG GAT GGC CTG GTG GAC CTG GCC GTG GGA GCC CAG GGG CAC Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln Gly His 595 600 605 610	1881
GTG CTG CTG CTT AGG AGT CTG CCT TTG CTG AAA GTG GGG ATC TCC ATT Val Leu Leu Leu Arg Ser Leu Pro Leu Leu Lys Val Gly Ile Ser Ile 615 620 625	1929
AGA TTT GCC CCC TCA GAG GTG GCA AAG ACT GTG TAC CAG TGC TGG GGA Arg Phe Ala Pro Ser Glu Val Ala Lys Thr Val Tyr Gln Cys Trp Gly 630 635 640	1977
AGG ACT CCC ACT GTC CTC GAA GCT GGA GAG GCC ACC GTC TGT CTC ACT Arg Thr Pro Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys Leu Thr 645 650 655	2025
GTC CGC AAA GGT TCA CCT GAC CTG TTA GGT GAT GTC CAA AGC TCT GTC Val Arg Lys Gly Ser Pro Asp Leu Leu Gly Asp Val Gln Ser Ser Val 660 665 670	2073
AGG TAT GAT CTG GCG TTG GAT CCG GGC CGT CTG ATT TCT CGT GCC ATT Arg Tyr Asp Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg Ala Ile 675 680 685 690	2121
TTT GAT GAG ACG AAG AAC TGC ACT TTG ACC CGA AGG AAG ACT CTG GGG Phe Asp Glu Thr Lys Asn Cys Thr Leu Thr Arg Arg Lys Thr Leu Gly 695 700 705	2169
CTT GGT GAT CAC TGC GAA ACA ATG AAG CTG CTT TTG CCA GAC TGT GTG Leu Gly Asp His Cys Glu Thr Met Lys Leu Leu Leu Pro Asp Cys Val 710 715 720	2217
GAG GAT GCA GTG ACC CCT ATC ATC CTG CGC CTT AAC TTA TCC CTG GCA Glu Asp Ala Val Thr Pro Ile Ile Leu Arg Leu Asn Leu Ser Leu Ala 725 730 735	2265
GGG GAC TCT GCT CCA TGC AGG AAC CTT CGT CCT GTG CTG GCT GTG GGC Gly Asp Ser Ala Pro Ser Arg Asn Leu Arg Pro Val Leu Ala Val Gly 740 745 750	2313
TCA CAA GAC CAT GTA ACA GCT TCT TTC CCG TTT GAG AAG AAC TGT GAG Ser Gln Asp His Val Thr Ala Ser Phe Pro Phe Glu Lys Asn Cys Glu 755 760 765 770	2361
GGG AAC CTG GGC GTC AGC TTC AAC TTC TCA GGC CTG CAG GTC TTG GAG Gly Asn Leu Gly Val Ser Phe Asn Phe Ser Gly Leu Gln Val Leu Glu 775 780 785	2409
GTA CGA AGC TCC CCA GAG CTC ACT GTG ACA GTA ACA GTT TGG AAT CAG Val Gly Ser Ser Pro Glu Leu Thr Val Thr Val Thr Val Trp Asn Glu 790 795 800	2457
GGT GAG GAC AGC TAT GGA ACC TTA ATC AAG TTC TAC TAC CCA GCA GAG Gly Glu Asp Ser Tyr Gly Thr Leu Ile Lys Phe Tyr Tyr Pro Ala Glu 805 810 815	2505

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CTA TCT TAC CGA CGG GTG ACA AGA GCC CAG CAA CCT CAT CCG TAC CCA Leu Ser Tyr Arg Arg Val Thr Arg Ala Gln Gln Pro His Pro Tyr Pro 820 825 830	2553
CTA CGC CTG GCA TGT GAG GCT GAG CCC ACG GGC CAG GAG AGC CTG AGG Leu Arg Leu Ala Cys Glu Ala Glu Pro Thr Gly Gln Glu Ser Leu Arg 835 840 845 850	2601
AGC AGC AGC TGT AGC ATC AAT CAC CCC ATC TTC CGA GAA GGT GCC AAG Ser Ser Ser Cys Ser Ile Asn His Pro Ile Phe Arg Glu Gly Ala Lys 855 860 865	2649
GCC ACC TTC ATG ATC ACA TTT GAT GTC TCC TAC AAG GCC TTC CTG GGA Ala Thr Phe Met Ile Thr Phe Asp Val Ser Tyr Lys Ala Phe Leu Gly 870 875 880	2697
GAC AGG TTG CTT CTG AGG GCC AGC GCA AGC AGT GAG AAT AAT AAG CCT Asp Arg Leu Leu Leu Arg Ala Ser Ala Ser Ser Glu Asn Asn Lys Pro 885 890 895	2745
GAA ACC ACC AAC ACT GCC TTC CAG CTG GAG CTT CCG GTG AAG TAC ACG Glu Thr Ser Lys Thr Ala Phe Gln Leu Glu Leu Pro Val Lys Tyr Thr 900 905 910	2793
GTC TAT ACC GTG ATC AGT AGG CAG GAA GAT TCT ACC AAG CAT TTC AAC Val Tyr Thr Val Ile Ser Arg Gln Glu Asp Ser Thr Lys His Phe Asn 915 920 925 930	2841
TTC TCA TCT TCC CAC GGG GAG AGA CAG AAA GAG GCC GAA CAT CGA TAT Phe Ser Ser Ser His Gly Glu Arg Gln Lys Glu Ala Glu His Arg Tyr 935 940 945	2889
CGT GTG AAT AAC CTG ACT CCA TTG ACG CTG GCC ATC AGC GTT AAC TTC Arg Val Asn Asn Leu Ser Pro Leu Thr Leu Ala Ile Ser Val Asn Phe 950 955 960	2937
TGG GTC CCC ATC CTT CTG AAT GGT GTG GCC GTG TGG GAT GTG ACT CTG Trp Val Pro Ile Leu Leu Asn Gly Val Ala Val Trp Asp Val Thr Leu 965 970 975	2985
AGG AGC CCA GCA CAG GGT GTC TCC TGT GTG TCA CAG AGG GAA CCT CCT Arg Ser Pro Ala Gln Gly Val Ser Cys Val Ser Gln Arg Glu Pro Pro 980 985 990	3033
CAA CAT TCC GAC CTT CTG ACC CAG ATC CAA GGA CGC TCT GTG CTG GAC Gln His Ser Asp Leu Leu Thr Gln Ile Gln Gly Arg Ser Val Leu Asp 995 1000 1005 1010	3081
TGC GCC ATC GCC GAC TGC CTG CAC CTC CGC TGT GAC ATC CCC TCC TTG Cys Ala Ile Ala Asp Cys Leu His Leu Arg Cys Asp Ile Pro Ser Leu 1015 1020 1025	3129
GGC ACC CTG GAT GAG CTT GAC TTC ATT CTG AAG GGC AAC CTC AGC TTC Gly Thr Leu Asp Glu Leu Asp Phe Ile Leu Lys Gly Asn Leu Ser Phe 1030 1035 1040	3177
GGC TGG ATC AGT CAG ACA TTG CAG AAA AAG GTG TTG CTC CTG AGT GAG Gly Trp Ile Ser Gln Thr Leu Gln Lys Lys Val Leu Leu Leu Ser Glu 1045 1050 1055	3225

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GCT GAA ATC ACA TTC AAC ACA TCT GTG TAT TCC CAG CTG CCG GGA CAG 3273  
 Ala Glu Ile Thr Phe Asn Thr Ser Val Tyr Ser Gln Leu Pro Gly Gln  
 1060 1065 1070

GAG GCA TTT CTG AGA GCC CAG GTG TCA ACG ATG CTA GAA GAA TAC GTG 3321  
 Glu Ala Phe Leu Arg Ala Gln Val Ser Thr Met Leu Glu Glu Tyr Val  
 1075 1080 1085 1090

GTC TAT GAG CCC GTC TTC CTC ATG GTG TTC AGC TCA GTG GGA GGT CTG 3369  
 Val Tyr Glu Pro Val Phe Leu Met Val Phe Ser Ser Val Gly Gly Leu  
 1095 1100 1105

CTG TTA CTG GCT CTC ATC ACT GTG GCG CTG TAC AAG CTT GGC TTC TTC 3417  
 Leu Leu Leu Ala Leu Ile Thr Val Ala Leu Tyr Lys Leu Gly Phe Phe  
 1110 1115 1120

AAA CGT CAG TAT AAA GAG ATG CTG GAT CTA CCA TCT GCA GAT CCT GAC 3465  
 Lys Arg Gln Tyr Lys Glu Met Leu Asp Leu Pro Ser Ala Asp Pro Asp  
 1125 1130 1135

CCA GCC GGC CAG GCA GAT TCC AAC CAT GAG ACT CCT CCA CAT CTC ACG 3513  
 Pro Ala Gly Gln Ala Asp Ser Asn His Glu Thr Pro Pro His Leu Thr  
 1140 1145 1150

TCC TAG 3519  
 Ser  
 1155

## (2) INFORMATION FOR SEQ ID NO:46:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1155 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Met Val Arg Gly Val Val Ile Leu Leu Cys Gly Trp Ala Leu Ala Ser  
 1 5 10 15

Cys His Gly Ser Asn Leu Asp Val Glu Lys Pro Val Val Phe Lys Glu  
 20 25 30

Asp Ala Ala Ser Phe Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg  
 35 40 45

Leu Val Val Gly Ala Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly  
 50 55 60

Gln Ser Ser Asp Cys Pro Pro Ala Thr Gly Val Cys Gln Pro Ile Leu  
 65 70 75 80

Leu His Ile Pro Leu Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu  
 85 90 95

Val Ala Asp Thr Asn Asn Ser Gln Leu Leu Ala Cys Gly Pro Thr Ala  
 100 105 110

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Gln Arg Ala Cys Ala Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu  
 115 120 125  
 Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala Ile Pro Ala Thr Met Pro  
 130 135 140  
 Glu Cys Pro Gly Gln Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser  
 145 150 155 160  
 Gly Ser Ile Asp Gln Ser Asp Phe Thr Gln Met Lys Asp Phe Val Lys  
 165 170 175  
 Ala Leu Met Gly Gln Leu Ala Ser Thr Ser Thr Ser Phe Ser Leu Met  
 180 185 190  
 Gln Tyr Ser Asn Ile Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys  
 195 200 205  
 Ser Ser Leu Ser Pro Gln Ser Leu Val Asp Ala Ile Val Gln Leu Gln  
 210 215 220  
 Gly Leu Thr Tyr Thr Ala Ser Gly Ile Gln Lys Val Val Lys Glu Leu  
 225 230 235 240  
 Phe His Ser Lys Asn Gly Ala Arg Lys Ser Ala Lys Lys Ile Leu Ile  
 245 250 255  
 Val Ile Thr Asp Gly Gln Lys Phe Arg Asp Pro Leu Glu Tyr Arg His  
 260 265 270  
 Val Ile Pro Glu Ala Glu Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly  
 275 280 285  
 Val Gly Asp Ala Phe Arg Glu Pro Thr Ala Leu Gln Glu Leu Asn Thr  
 290 295 300  
 Ile Gly Ser Ala Pro Ser Gln Asp His Val Phe Lys Val Gly Asn Phe  
 305 310 315 320  
 Val Ala Leu Arg Ser Ile Gln Arg Gln Ile Gln Glu Lys Ile Phe Ala  
 325 330 335  
 Ile Glu Gly Thr Glu Ser Arg Ser Ser Ser Ser Phe Gln His Glu Met  
 340 345 350  
 Ser Gln Glu Gly Phe Ser Ser Ala Leu Ser Met Asp Gly Pro Val Leu  
 355 360 365  
 Gly Ala Val Gly Gly Phe Ser Trp Ser Gly Gly Ala Phe Leu Tyr Pro  
 370 375 380  
 Ser Asn Met Arg Ser Thr Phe Ile Asn Met Ser Gln Glu Asn Glu Asp  
 385 390 395 400  
 Met Arg Asp Ala Tyr Leu Gly Tyr Ser Thr Ala Leu Ala Phe Trp Lys  
 405 410 415  
 Gly Val His Ser Leu Ile Leu Gly Ala Pro Arg His Gln His Thr Gly  
 420 425 430



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Lys Val Val Ile Phe Thr Gln Glu Ser Arg His Trp Arg Pro Lys Ser  
 435 440 445  
 Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys  
 450 455 460  
 Ser Val Asp Met Asp Arg Asp Gly Ser Thr Asp Leu Val Leu Ile Gly  
 465 470 475 480  
 Val Pro His Tyr Tyr Glu His Thr Arg Gly Gly Gln Val Ser Val Cys  
 485 490 495  
 Pro Met Pro Gly Val Arg Ser Arg Trp His Cys Gly Thr Thr Leu His  
 500 505 510  
 Gly Glu Gln Gly His Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val  
 515 520 525  
 Leu Gly Asp Val Asn Gly Asp Ser Leu Ala Asp Val Ala Ile Gly Ala  
 530 535 540  
 Pro Gly Glu Glu Glu Asn Arg Gly Ala Val Tyr Ile Phe His Gly Ala  
 545 550 555 560  
 Ser Arg Gln Asp Ile Ala Pro Ser Pro Ser Gln Arg Val Thr Gly Ser  
 565 570 575  
 Gln Leu Phe Leu Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly  
 580 585 590  
 Gln Asp Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln  
 595 600 605  
 Gly His Val Leu Leu Leu Arg Ser Leu Pro Leu Leu Lys Val Gly Ile  
 610 615 620  
 Ser Ile Arg Phe Ala Pro Ser Glu Val Ala Lys Thr Val Tyr Gln Cys  
 625 630 635 640  
 Trp Gly Arg Thr Pro Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys  
 645 650 655  
 Leu Thr Val Arg Lys Gly Ser Pro Asp Leu Leu Gly Asp Val Gln Ser  
 660 665 670  
 Ser Val Arg Tyr Asp Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg  
 675 680 685  
 Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr Leu Thr Arg Arg Lys Thr  
 690 695 700  
 Leu Gly Leu Gly Asp His Cys Glu Thr Met Lys Leu Leu Leu Pro Asp  
 705 710 715 720  
 Cys Val Glu Asp Ala Val Thr Pro Ile Ile Leu Arg Leu Asn Leu Ser  
 725 730 735  
 Leu Ala Gly Asp Ser Ala Pro Ser Arg Asn Leu Arg Pro Val Leu Ala  
 740 745 750

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Val Gly Ser Gln Asp His Val Thr Ala Ser Phe Pro Phe Glu Lys Asn  
 755 760 765  
 Cys Glu Gly Asn Leu Gly Val Ser Phe Asn Phe Ser Gly Leu Gln Val  
 770 775 780  
 Leu Glu Val Gly Ser Ser Pro Glu Leu Thr Val Thr Val Thr Val Trp  
 785 790 795 800  
 Asn Glu Gly Glu Asp Ser Tyr Gly Thr Leu Ile Lys Phe Tyr Tyr Pro  
 805 810 815  
 Ala Glu Leu Ser Tyr Arg Arg Val Thr Arg Ala Gln Gln Pro His Pro  
 820 825 830  
 Tyr Pro Leu Arg Leu Ala Cys Glu Ala Glu Pro Thr Gly Gln Glu Ser  
 835 840 845  
 Leu Arg Ser Ser Ser Cys Ser Ile Asn His Pro Ile Phe Arg Glu Gly  
 850 855 860  
 Ala Lys Ala Thr Phe Met Ile Thr Phe Asp Val Ser Tyr Lys Ala Phe  
 865 870 875 880  
 Leu Gly Asp Arg Leu Leu Leu Arg Ala Ser Ala Ser Ser Glu Asn Asn  
 885 890 895  
 Lys Pro Glu Thr Ser Lys Thr Ala Phe Gln Leu Glu Leu Pro Val Lys  
 900 905 910  
 Tyr Thr Val Tyr Thr Val Ile Ser Arg Gln Glu Asp Ser Thr Lys His  
 915 920 925  
 Phe Asn Phe Ser Ser Ser His Gly Glu Arg Gln Lys Glu Ala Glu His  
 930 935 940  
 Arg Tyr Arg Val Asn Asn Leu Ser Pro Leu Thr Leu Ala Ile Ser Val  
 945 950 955 960  
 Asn Phe Trp Val Pro Ile Leu Leu Asn Gly Val Ala Val Trp Asp Val  
 965 970 975  
 Thr Leu Arg Ser Pro Ala Gln Gly Val Ser Cys Val Ser Gln Arg Glu  
 980 985 990  
 Pro Pro Gln His Ser Asp Leu Leu Thr Gln Ile Gln Gly Arg Ser Val  
 995 1000 1005  
 Leu Asp Cys Ala Ile Ala Asp Cys Leu His Leu Arg Cys Asp Ile Pro  
 1010 1015 1020  
 Ser Leu Gly Thr Leu Asp Glu Leu Asp Phe Ile Leu Lys Gly Asn Leu  
 1025 1030 1035 1040  
 Ser Phe Gly Trp Ile Ser Gln Thr Leu Gln Lys Lys Val Leu Leu Leu  
 1045 1050 1055  
 Ser Glu Ala Glu Ile Thr Phe Asn Thr Ser Val Tyr Ser Gln Leu Pro  
 1060 1065 1070

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Gly Gln Glu Ala Phe Leu Arg Ala Gln Val Ser Thr Met Leu Glu Glu  
 1075 1080 1085

Tyr Val Val Tyr Glu Pro Val Phe Leu Met Val Phe Ser Ser Val Gly  
 1090 1095 1100

Gly Leu Leu Leu Leu Ala Leu Ile Thr Val Ala Leu Tyr Lys Leu Gly  
 1105 1110 1115 1120

Phe Phe Lys Arg Gln Tyr Lys Glu Met Leu Asp Leu Pro Ser Ala Asp  
 1125 1130 1135

Pro Asp Pro Ala Gly Gln Ala Asp Ser Asn His Glu Thr Pro Pro His  
 1140 1145 1150

Leu Thr Ser  
 1155

## (2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 49 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AGTTACGGAT CCGGCACCAT GACCTTCGGC ACTGTGATCC TCCTGTGTG

49

## (2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 19 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCTGGACGAT GGCATCCAC

19

## (2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GTAGAGTTAC GGATCCGGCA CCAT

24

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCAGCCAGCT TCGGACAGAC

20

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CCATGTCCAC AGAACAGAGA G

21

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3803 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..3486

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATG	GTC	CGT	GGA	GTT	GTG	ATC	CTC	CTG	TGT	GGC	TGG	GCC	CTG	GCT	TCC	48
Met	Val	Arg	Gly	Val	Val	Ile	Leu	Leu	Cys	Gly	Trp	Ala	Leu	Ala	Ser	
1				5				10					15			
TGT	CAT	GGG	TCT	AAC	CTG	GAT	GTG	GAG	AAG	CCC	GTC	GTG	TTC	AAA	GAG	96
Cys	His	Gly	Ser	Asn	Leu	Asp	Val	Glu	Lys	Pro	Val	Val	Phe	Lys	Glu	
				20				25					30			

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GAT GCA GCC AGC TTC GGA CAG ACT GTG GTG CAG TTT GGT GGA TCT CGA Asp Ala Ala Ser Phe Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg 35 40 45	144
CTC GTG GTG GGA GCC CCT CTG GAG GCG GTG GCA GTC AAC CAA ACA GGA Leu Val Val Gly Ala Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly 50 55 60	192
CAG TCG TCT GAC TGT CCG CCT GCC ACT GGC GTG TGC CAG CCC ATC TTA Gln Ser Ser Asp Cys Pro Ala Thr Gly Val Cys Gln Pro Ile Leu 65 70 75 80	240
CTG CAC ATT CCC CTA GAG GCA GTG AAC ATG TCC CTG GGC CTG TCT CTG Leu His Ile Pro Leu Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu 85 90 95	288
GTG GCT GAC ACC AAT AAC TCC CAG TTG CTG GCT TGT GGT CCA ACT GCA Val Ala Asp Thr Asn Asn Ser Gln Leu Leu Ala Cys Gly Pro Thr Ala 100 105 110	336
CAG AGA GCT TGT GCA AAG AAC ATG TAT GCA AAA GGT TCC TGC CTC CTT Gln Arg Ala Cys Ala Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu 115 120 125	384
CTG GGC TCC AGC TTG CAG TTC ATC CAG GCA ATC CCT GCT ACC ATG CCA Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala Ile Pro Ala Thr Met Pro 130 135 140	432
GAG TGT CCA GGA CAA GAG ATG GAC ATT GCT TTC CTG ATT GAT GGC TCC Glu Cys Pro Gly Gln Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser 145 150 155 160	480
GGC AGC ATT GAT CAA AGT GAC TTT ACC CAG ATG ARG GAC TTC GTC AAA Gly Ser Ile Asp Gln Ser Asp Phe Thr Gln Met Lys Asp Phe Val Lys 165 170 175	528
GCT TTG ATG GGC CAG TTG GCG AGC ACC AGC ACC TCG TTC TCC CTG ATG Ala Leu Met Gly Gln Leu Ala Ser Thr Ser Thr Ser Phe Ser Leu Met 180 185 190	576
CAA TAC TCA AAC ATC CTG AAG ACT CAT TTT ACC TTC ACG GAA TTC AAG Gln Tyr Ser Asn Ile Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys 195 200 205	624
AGC AGC CTG AGC CCT CAG AGC CTG GTG GAT GCC ATC GTC CAG CTC CAA Ser Ser Leu Ser Pro Gln Ser Leu Val Asp Ala Ile Val Gln Leu Gln 210 215 220	672
GGC CTG ACG TAC ACA GCC TCG GGC ATC CAG AAA GTG GTG AAA GAG CTA Gly Leu Thr Tyr Thr Ala Ser Gly Ile Gln Lys Val Val Lys Glu Leu 225 230 235 240	720
TTT CAT AGC AAG AAT GGG GCC CGA AAA AGT GCC AAG AAG ATA CTA ATT Phe His Ser Lys Asn Gly Ala Arg Lys Ser Ala Lys Lys Ile Leu Ile 245 250 255	768
GTC ATC ACA GAT GGG CAG AAA TTC AGA GAC CCC CTG GAG TAT AGA CAT Val Ile Thr Asp Gly Gln Lys Phe Arg Asp Pro Leu Glu Tyr Arg His 260 265 270	816

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GTC ATC CCT GAA GCA GAG AAA GCT GGG ATC ATT CGC TAT GCT ATA GGG Val Ile Pro Glu Ala Glu Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly 275 280 285	864
GTG GGA GAT GCC TTC CGG GAA CCC ACT GCC CTA CAG GAG CTG AAC ACC Val Gly Asp Ala Phe Arg Glu Pro Thr Ala Leu Gln Glu Leu Asn Thr 290 295 300	912
ATT GGC TCA GCT CCC TCG CAG GAC CAC GTG TTC AAG GTG GGC AAT TTT Ile Gly Ser Ala Pro Ser Gln Asp His Val Phe Lys Val Gly Asn Phe 305 310 315 320	960
GTA GCA CTT CGC AGC ATC CAG CGG CAA ATT CAG GAG AAA ATC TTT GCC Val Ala Leu Arg Ser Ile Gln Arg Gln Ile Gln Glu Lys Ile Phe Ala 325 330 335	1008
ATT GAA GGA ACC GAA TCA AGG TCA AGT AGT TCC TTT CAG CAC GAG ATG Ile Glu Gly Thr Glu Ser Arg Ser Ser Ser Ser Phe Gln His Glu Met 340 345 350	1056
TCA CAA GAA GGT TTC AGC TCA GCT CTC TCA ATG GAT GGA CCA GTT CTG Ser Gln Glu Gly Phe Ser Ser Ala Leu Ser Met Asp Gly Pro Val Leu 355 360 365	1104
GGG GCT GTG GGA GGC TTC AGC TGG TCT GGA GGT GCC TTC TTG TAC CCC Gly Ala Val Gly Gly Phe Ser Trp Ser Gly Gly Ala Phe Leu Tyr Pro 370 375 380	1152
TCA AAT ATG AGA TCC ACC TTC ATC AAC ATG TCT CAG GAG AAC GAG GAT Ser Asn Met Arg Ser Thr Phe Ile Asn Met Ser Gln Glu Asn Glu Asp 385 390 395 400	1200
ATG AGG GAC GCT TAC CTG GGT TAC TCC ACC GCA CTG GCC TTT TGG AAG Met Arg Asp Ala Tyr Leu Gly Tyr Ser Thr Ala Leu Ala Phe Trp Lys 405 410 415	1248
GGG GTC CAC AGC CTG ATC CTG GGG GCC CCT CGC CAC CAG CAC ACG GGG Gly Val His Ser Leu Ile Leu Gly Ala Pro Arg His Gln His Thr Gly 420 425 430	1296
AAG GTT GTC ATC TTT ACC CAG GAA TCC AGG CAC TGG AGG CCC AAG TCT Lys Val Val Ile Phe Thr Gln Glu Ser Arg His Trp Arg Pro Lys Ser 435 440 445	1344
GAA GTC AGA GGG ACA CAG ATC GGC TCC TAC TTT GGG GCA TCT CTC TGT Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys 450 455 460	1392
TCT GTG GAC ATG GAT AGA GAT GGC AGC ACT GAC CTC CTC CTG ATT GGA Ser Val Asp Met Asp Arg Asp Gly Ser Thr Asp Leu Val Leu Ile Gly 465 470 475 480	1440
GTC CCC CAT TAC TAT GAG CAC ACC CGA GGG GGG CAG GTG TCG GTG TGC Val Pro His Tyr Tyr Glu His Thr Arg Gly Gly Gln Val Ser Val Cys 485 490 495	1488
CCC ATG CCT GGT GTG AGG AGC AGG TGG CAT TGT GGG ACC ACC CTC CAT Pro Met Pro Gly Val Arg Ser Arg Trp His Cys Gly Thr Thr Leu His 500 505 510	1536

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GGG GAG CAG GGC CAT CCT TGG GGC CGC TTT GGG GCG GCT CTG ACA GTG Gly Glu Gln Gly His Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val 515 520 525	1584
CTA GGG GAC GTG AAT GGG GAC AGT CTG GCG GAT GTG GCT ATT GGT GCA Leu Gly Asp Val Asn Gly Asp Ser Leu Ala Asp Val Ala Ile Gly Ala 530 535 540	1632
CCC GCA GAG GAG GAG AAC AGA GGT GCT GTC TAC ATA TTT CAT GGA GCC Pro Gly Glu Glu Glu Asn Arg Gly Ala Val Tyr Ile Phe His Gly Ala 545 550 555 560	1680
TCG AGA CAG GAC ATC GCT CCC TCG CCT AGC CAG CGG GTC ACT GGC TCC Ser Arg Gln Asp Ile Ala Pro Ser Pro Ser Gln Arg Val Thr Gly Ser 565 570 575	1728
CAG CTC TTC CTG AGC CTC CAA TAT TTT GGG CAG TCA TTA AGT GGG GGT Gln Leu Phe Leu Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly 580 585 590	1776
CAG GAC CTT ACA CAG GAT GGC CTG GTG GAC CTG GCC GTG GGA GCC CAG Gln Asp Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln 595 600 605	1824
GGG CAC GTG CTG CTG CTT AGG AGT CTG CCT TTG CTG AAA GTG GGG ATC Gly His Val Leu Leu Leu Arg Ser Leu Pro Leu Leu Lys Val Gly Ile 610 615 620	1872
TCC ATT AGA TTT GCC CCC TCA GAG GTG GCA AAG ACT GTG TAC CAG TGC Ser Ile Arg Phe Ala Pro Ser Glu Val Ala Lys Thr Val Tyr Gln Cys 625 630 635 640	1920
TGG GGA AGG ACT CCC ACT GTC CTC GAA GCT GGA GAG GCC ACC GTC TGT Trp Gly Arg Thr Pro Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys 645 650 655	1968
CTC ACT GTC CGC AAA GGT TCA CCT GAC CTG TTA GGT GAT GTC CAA AGC Leu Thr Val Arg Lys Gly Ser Pro Asp Leu Leu Gly Asp Val Gln Ser 660 665 670	2016
TCT GTC AGG TAT GAT CTG GCG TTG GAT CCG GGC CGT CTG ATT TCT CGT Ser Val Arg Tyr Asp Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg 675 680 685	2064
GCC ATT TTT GAT GAG ACG AAG AAC TGC ACT TTG ACC CGA AGG AAG ACT Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr Leu Thr Arg Arg Lys Thr 690 695 700	2112
CTG GGG CTT GGT GAT CAC TGC GAA ACA ATG AAG CTG CTT TTG CCA GAC Leu Gly Leu Gly Asp His Cys Glu Thr Met Lys Leu Leu Leu Pro Asp 705 710 715 720	2160
TGT GTG GAG GAT GCA GTG ACC CCT ATC ATC CTG CGC CTT AAC TTA TCC Cys Val Glu Asp Ala Val Thr Pro Ile Ile Leu Arg Leu Asn Leu Ser 725 730 735	2208
CTG GCA GGG GAC TCT GCT CCA TCC AGG AAC CTT CGT CCT GTG CTG GCT Leu Ala Gly Asp Ser Ala Pro Ser Arg Asn Leu Arg Pro Val Leu Ala 740 745 750	2256

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GTG GGC TCA CAA GAC CAT GTA ACA GCT TCT TTC CCG TTT GAG AAG AAC Val Gly Ser Gln Asp His Val Thr Ala Ser Phe Pro Phe Glu Lys Asn 755 760 765	2304
TGT AAG CAG GAG CTC CTG TGT GAG GGG AAC CTG GGC GTC AGC TTC AAC Cys Lys Gln Glu Leu Leu Cys Glu Gly Asn Leu Gly Val Ser Phe Asn 770 775 780	2352
TTC TCA GGC CTG CAG GTC TTG GAG GTA GGA AGC TCC CCA GAG CTC ACT Phe Ser Gly Leu Gln Val Leu Glu Val Gly Ser Ser Pro Glu Leu Thr 785 790 795 800	2400
GTG ACA GTA ACA GTT TGG AAT GAG GGT GAG GAC AGC TAT GGA ACC TTA Val Thr Val Thr Val Trp Asn Glu Gly Glu Asp Ser Tyr Gly Thr Leu 805 810 815	2448
ATC AAG TTC TAC TAC CCA GCA GAG CTA TCT TAC CGA CGG GTG ACA AGA Ile Lys Phe Tyr Tyr Pro Ala Glu Leu Ser Tyr Arg Arg Val Thr Arg 820 825 830	2496
GCC CAG CAA CCT CAT CCG TAC CCA CTA CGC CTG GCA TGT GAG GCT GAG Ala Gln Gln Pro His Pro Tyr Pro Leu Arg Leu Ala Cys Glu Ala Glu 835 840 845	2544
CCC ACG GGC CAG GAG AGC CTG AGG AGC AGC AGC TGT AGC ATC AAT CAC Pro Thr Gly Gln Glu Ser Leu Arg Ser Ser Ser Cys Ser Ile Asn His 850 855 860	2592
CCC ATC TTC CGA GAA GGT GCC AAG GCC ACC TTC ATG ATC ACA TTT GAT Pro Ile Phe Arg Glu Gly Ala Lys Ala Thr Phe Met Ile Thr Phe Asp 865 870 875 880	2640
CTC TCC TAC AAG GCC TTC CTG GGA GAC AGG TTG CTT CTG AGG GCC AGC Val Ser Tyr Lys Ala Phe Leu Gly Asp Arg Leu Leu Leu Arg Ala Ser 885 890 895	2688
GCA AGC AGT GAG AAT AAT AAG CCT GAA ACC AGC AAG ACT GCC TTC CAG Ala Ser Ser Glu Asn Asn Lys Pro Glu Thr Ser Lys Thr Ala Phe Gln 900 905 910	2736
CTG GAG CTT CCG GTG AAG TAC ACG GTC TAT ACC GTG ATC AGT AGG CAG Leu Glu Leu Pro Val Lys Tyr Thr Val Tyr Thr Val Ile Ser Arg Gln 915 920 925	2784
GAA GAT TCT ACC AAG CAT TTC AAC TTC TCA TCT TCC CAC GGG GAG AGA Glu Asp Ser Thr Lys His Phe Asn Phe Ser Ser Ser His Gly Glu Arg 930 935 940	2832
CAG AAA GAG GCC GAA CAT CGA TAT CGT GTG AAT AAC CTG AGT CCA TTG Gln Lys Glu Ala Glu His Arg Tyr Arg Val Asn Asn Leu Ser Pro Leu 945 950 955 960	2880
ACG CTG GCC ATC AGC GTT AAC TTC TGG GTC CCC ATC CTT CTG AAT GGT Thr Leu Ala Ile Ser Val Asn Phe Trp Val Pro Ile Leu Leu Asn Gly 965 970 975	2928
TGG GCC GTG TGG GAT GTG ACT CTG AGG AGC CCA GCA CAG GGT GTC TCC Ala Ala Val Trp Asp Val Thr Leu Arg Ser Pro Ala Gln Gly Val Ser 980 985 990	2976



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TGT GTG TCA CAG AGG GAA CCT CCT CAA CAT TCC GAC CTT CTG ACC CAG Cys Val Ser Gln Arg Glu Pro Pro Gln His Ser Asp Leu Leu Thr Gln 995 1000 1005	3024
ATC CAA GGA CGC TCT GTG CTG GAC TGC GCC ATC GCC GAC TGC CTG CAC Ile Gln Gly Arg Ser Val Leu Asp Cys Ala Ile Ala Asp Cys Leu His 1010 1015 1020	3072
CTC CGC TGT GAC ATC CCC TCC TTG GGC ACC CTG GAT GAG CTT GAC TTC Leu Arg Cys Asp Ile Pro Ser Leu Gly Thr Leu Asp Glu Leu Asp Phe 1025 1030 1035 1040	3120
ATT CTG AAG GGC AAC CTC AGC TTC GGC TGG ATC AGT CAG ACA TTG CAG Ile Leu Lys Gly Asn Leu Ser Phe Gly Trp Ile Ser Gln Thr Leu Gln 1045 1050 1055	3168
AAA AAG GTG TTG CTC CTG AGT GAG GCT GAA ATC ACA TTC AAC ACA TCT Lys Lys Val Leu Leu Leu Ser Glu Ala Glu Ile Thr Phe Asn Thr Ser 1060 1065 1070	3216
GTG TAT TCC CAG CTG CCG GGA CAG GAG GCA TTT CTG AGA GCC CAG GTG Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala Phe Leu Arg Ala Gln Val 1075 1080 1085	3264
TCA ACG ATG CTA GAA GAA TAC GTG GTC TAT GAG CCC GTC TTC CTC ATG Ser Thr Met Leu Glu Glu Tyr Val Val Tyr Glu Pro Val Phe Leu Met 1090 1095 1100	3312
GTG TTC AGC TCA GTG GGA GGT CTG CTG TTA CTG GCT CTC ATC ACT GTG Val Phe Ser Ser Val Gly Gly Leu Leu Leu Leu Ala Leu Ile Thr Val 1105 1110 1115 1120	3360
GCG CTG TAC AAG CTT GGC TTC TTC AAA CGT CAG TAT AAA GAG ATG CTG Ala Leu Tyr Lys Leu Gly Phe Phe Lys Arg Gln Tyr Lys Glu Met Leu 1125 1130 1135	3408
GAT CTA CCA TCT GCA GAT CCT GAC CCA GCC GGC CAG GCA GAT TCC AAC Asp Leu Pro Ser Ala Asp Pro Asp Pro Ala Gly Gln Ala Asp Ser Asn 1140 1145 1150	3456
CAT GAG ACT CCT CCA CAT CTC ACG TCC TAGGAATCTA CTTTCCTGTA His Glu Thr Pro Pro His Leu Thr Ser 1155 1160	3503
TATCTCCACA ATTACGAGAT TGTTTTTGCT TTTGCCTATG AATCTACTGG CATGGGAACA	3563
AGTTCTCTTC AGCTCTGGGC TAGCCTGGGA AACTTCCCAG AAATGATGCC CTACCTCCTG	3623
AGCTGGGAGA TTTTATGGT TTGCCCATGT GTCAGATTTC AGTGCTGATC CACTTTTTTT	3683
GCAAGAGCAG GAATGGGGTC AGCATAAATT TACATATGGA TAAGAACTAA CACAAGACTG	3743
AGTAATATGC TCAATATTCA ATGTATTGCT TGTATAAATT TTTAAAAAAT AAAATGAAAN	3803

## (2) INFORMATION FOR SEQ ID NO:53:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1161 amino acids  
(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```

Met Val Arg Gly Val Val Ile Leu Leu Cys Gly Trp Ala Leu Ala Ser
 1           5           10           15
Cys His Gly Ser Asn Leu Asp Val Glu Lys Pro Val Val Phe Lys Glu
 20           25           30
Asp Ala Ala Ser Phe Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg
 35           40           45
Leu Val Val Gly Ala Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly
 50           55           60
Gln Ser Ser Asp Cys Pro Pro Ala Thr Gly Val Cys Gln Pro Ile Leu
 65           70           75           80
Leu His Ile Pro Leu Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu
 85           90           95
Val Ala Asp Thr Asn Asn Ser Gln Leu Leu Ala Cys Gly Pro Thr Ala
100          105          110
Gln Arg Ala Cys Ala Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu
115          120          125
Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala Ile Pro Ala Thr Met Pro
130          135          140
Glu Cys Pro Gly Gln Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser
145          150          155          160
Gly Ser Ile Asp Gln Ser Asp Phe Thr Gln Met Lys Asp Phe Val Lys
165          170          175
Ala Leu Met Gly Gln Leu Ala Ser Thr Ser Thr Ser Phe Ser Leu Met
180          185          190
Gln Tyr Ser Asn Ile Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys
195          200          205
Ser Ser Leu Ser Pro Gln Ser Leu Val Asp Ala Ile Val Gln Leu Gln
210          215          220
Gly Leu Thr Tyr Thr Ala Ser Gly Ile Gln Lys Val Val Lys Glu Leu
225          230          235          240
Phe His Ser Lys Asn Gly Ala Arg Lys Ser Ala Lys Lys Ile Leu Ile
245          250          255
Val Ile Thr Asp Gly Gln Lys Phe Arg Asp Pro Leu Glu Tyr Arg His
260          265          270
Val Ile Pro Glu Ala Glu Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly
275          280          285

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Val Gly Asp Ala Phe Arg Glu Pro Thr Ala Leu Gln Glu Leu Asn Thr  
 290 295 300  
 Ile Gly Ser Ala Pro Ser Gln Asp His Val Phe Lys Val Gly Asn Phe  
 305 310 315 320  
 Val Ala Leu Arg Ser Ile Gln Arg Gln Ile Gln Glu Lys Ile Phe Ala  
 325 330 335  
 Ile Glu Gly Thr Glu Ser Arg Ser Ser Ser Phe Gln His Glu Met  
 340 345 350  
 Ser Gln Glu Gly Phe Ser Ser Ala Leu Ser Met Asp Gly Pro Val Leu  
 355 360 365  
 Gly Ala Val Gly Gly Phe Ser Trp Ser Gly Gly Ala Phe Leu Tyr Pro  
 370 375 380  
 Ser Asn Met Arg Ser Thr Phe Ile Asn Met Ser Gln Glu Asn Glu Asp  
 385 390 395 400  
 Met Arg Asp Ala Tyr Leu Gly Tyr Ser Thr Ala Leu Ala Phe Trp Lys  
 405 410 415  
 Gly Val His Ser Leu Ile Leu Gly Ala Pro Arg His Gln His Thr Gly  
 420 425 430  
 Lys Val Val Ile Phe Thr Gln Glu Ser Arg His Trp Arg Pro Lys Ser  
 435 440 445  
 Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys  
 450 455 460  
 Ser Val Asp Met Asp Arg Asp Gly Ser Thr Asp Leu Val Leu Ile Gly  
 465 470 475 480  
 Val Pro His Tyr Tyr Glu His Thr Arg Gly Gly Gln Val Ser Val Cys  
 485 490 495  
 Pro Met Pro Gly Val Arg Ser Arg Trp His Cys Gly Thr Thr Leu His  
 500 505 510  
 Gly Glu Gln Gly His Pro Trp Gly Arg Phe Gly Ala Ala Leu Thr Val  
 515 520 525  
 Leu Gly Asp Val Asn Gly Asp Ser Leu Ala Asp Val Ala Ile Gly Ala  
 530 535 540  
 Pro Gly Glu Glu Glu Asn Arg Gly Ala Val Tyr Ile Phe His Gly Ala  
 545 550 555 560  
 Ser Arg Gln Asp Ile Ala Pro Ser Pro Ser Gln Arg Val Thr Gly Ser  
 565 570 575  
 Gln Leu Phe Leu Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly  
 580 585 590  
 Gln Asp Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln  
 595 600 605

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Gly His Val Leu Leu Leu Arg Ser Leu Pro Leu Leu Lys Val Gly Ile  
 610 615 620  
 Ser Ile Arg Phe Ala Pro Ser Glu Val Ala Lys Thr Val Tyr Gln Cys  
 625 630 635 640  
 Trp Gly Arg Thr Pro Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys  
 645 650 655  
 Leu Thr Val Arg Lys Gly Ser Pro Asp Leu Leu Gly Asp Val Gln Ser  
 660 665 670  
 Ser Val Arg Tyr Asp Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg  
 675 680 685  
 Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr Leu Thr Arg Arg Lys Thr  
 690 695 700  
 Leu Gly Leu Gly Asp His Cys Glu Thr Met Lys Leu Leu Leu Pro Asp  
 705 710 715 720  
 Cys Val Glu Asp Ala Val Thr Pro Ile Ile Leu Arg Leu Asn Leu Ser  
 725 730 735  
 Leu Ala Gly Asp Ser Ala Pro Ser Arg Asn Leu Arg Pro Val Leu Ala  
 740 745 750  
 Val Gly Ser Gln Asp His Val Thr Ala Ser Phe Pro Phe Glu Lys Asn  
 755 760 765  
 Cys Lys Gln Glu Leu Leu Cys Glu Gly Asn Leu Gly Val Ser Phe Asn  
 770 775 780  
 Phe Ser Gly Leu Gln Val Leu Glu Val Gly Ser Ser Pro Glu Leu Thr  
 785 790 795 800  
 Val Thr Val Thr Val Trp Asn Glu Gly Glu Asp Ser Tyr Gly Thr Leu  
 805 810 815  
 Ile Lys Phe Tyr Tyr Pro Ala Glu Leu Ser Tyr Arg Arg Val Thr Arg  
 820 825 830  
 Ala Gln Gln Pro His Pro Tyr Pro Leu Arg Leu Ala Cys Glu Ala Glu  
 835 840 845  
 Pro Thr Gly Gln Glu Ser Leu Arg Ser Ser Ser Cys Ser Ile Asn His  
 850 855 860  
 Pro Ile Phe Arg Glu Gly Ala Lys Ala Thr Phe Met Ile Thr Phe Asp  
 865 870 875 880  
 Val Ser Tyr Lys Ala Phe Leu Gly Asp Arg Leu Leu Leu Arg Ala Ser  
 885 890 895  
 Ala Ser Ser Glu Asn Asn Lys Pro Glu Thr Ser Lys Thr Ala Phe Gln  
 900 905 910  
 Leu Glu Leu Pro Val Lys Tyr Thr Val Tyr Thr Val Ile Ser Arg Gln  
 915 920 925

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Glu Asp Ser Thr Lys His Phe Asn Phe Ser Ser Ser His Gly Glu Arg  
 930 935 940  
 Gln Lys Glu Ala Glu His Arg Tyr Arg Val Asn Asn Leu Ser Pro Leu  
 945 950 955 960  
 Thr Leu Ala Ile Ser Val Asn Phe Trp Val Pro Ile Leu Leu Asn Gly  
 965 970 975  
 Val Ala Val Trp Asp Val Thr Leu Arg Ser Pro Ala Gln Gly Val Ser  
 980 985 990  
 Cys Val Ser Gln Arg Glu Pro Pro Gln His Ser Asp Leu Leu Thr Gln  
 995 1000 1005  
 Ile Gln Gly Arg Ser Val Leu Asp Cys Ala Ile Ala Asp Cys Leu His  
 1010 1015 1020  
 Leu Arg Cys Asp Ile Pro Ser Leu Gly Thr Leu Asp Glu Leu Asp Phe  
 1025 1030 1035 1040  
 Ile Leu Lys Gly Asn Leu Ser Phe Gly Trp Ile Ser Gln Thr Leu Gln  
 1045 1050 1055  
 Lys Lys Val Leu Leu Leu Ser Glu Ala Glu Ile Thr Phe Asn Thr Ser  
 1060 1065 1070  
 Val Tyr Ser Gln Leu Pro Gly Gln Glu Ala Phe Leu Arg Ala Gln Val  
 1075 1080 1085  
 Ser Thr Met Leu Glu Glu Tyr Val Val Tyr Glu Pro Val Phe Leu Met  
 1090 1095 1100  
 Val Phe Ser Ser Val Gly Gly Leu Leu Leu Leu Ala Leu Ile Thr Val  
 1105 1110 1115 1120  
 Ala Leu Tyr Lys Leu Gly Phe Phe Lys Arg Gln Tyr Lys Glu Met Leu  
 1125 1130 1135  
 Asp Leu Pro Ser Ala Asp Pro Asp Pro Ala Gly Gln Ala Asp Ser Asn  
 1140 1145 1150  
 His Glu Thr Pro Pro His Leu Thr Ser  
 1155 1160

## (2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3597 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 40..3525

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AGCTTTACAG CTCTCTACTT CTCAGTGCAC TGCTCAGTG ATG GCC GGT GGA GTT	54
Met Ala Gly Gly Val	
1 5	
GTG ATC CTC CTG TGT GGC TGG GTC CTG GCT TCC TGT CAT GGG TCT AAC	102
Val Ile Leu Leu Cys Gly Trp Val Leu Ala Ser Cys His Gly Ser Asn	
10 15 20	
CTG CAT GTG GAG GAA CCC ATC GTG TTC AGA GAG GAT GCA GCC AGC TTT	150
Leu Asp Val Glu Glu Pro Ile Val Phe Arg Glu Asp Ala Ala Ser Phe	
25 30 35	
GGA CAG ACT GTG GTG CAG TTT GGT GGA TCT CGA CTC GTG GTG GGA GCC	198
Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg Leu Val Val Gly Ala	
40 45 50	
CCT CTG GAG GCG GTG GCA GTC AAC CAA ACA GGA CGG TTG TAT GAC TGT	246
Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly Arg Leu Tyr Asp Cys	
55 60 65	
GCA CCT GCC ACT GGC ATG TGC CAG CCC ATC GTA CTG CGC AGT CCC CTA	294
Ala Pro Ala Thr Gly Met Cys Gln Pro Ile Val Leu Arg Ser Pro Leu	
70 75 80 85	
GAG GCA GTG AAC ATG TCC CTG GGC CTG TCT CTG GTG ACT GCC ACC AAT	342
Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu Val Thr Ala Thr Asn	
90 95 100	
AAC GCC CAG TTG CTG GCT TGT GGT CCA ACT GCA CAG AGA GCT TGT GTG	390
Asn Ala Gln Leu Leu Ala Cys Gly Pro Thr Ala Gln Arg Ala Cys Val	
105 110 115	
AAG AAC ATG TAT GCG AAA GGT TCC TGC CTC CTT CTC GGC TCC AGC TTG	438
Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu Leu Gly Ser Ser Leu	
120 125 130	
CAG TTC ATC CAG GCA GTC CCT GCC TCC ATG CCA GAG TGT CCA AGA CAA	486
Gln Phe Ile Gln Ala Val Pro Ala Ser Met Pro Glu Cys Pro Arg Gln	
135 140 145	
GAG ATG GAC ATT GCT TTC CTG ATT GAT GGT TCT GGC AGC ATT AAC CAA	534
Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser Gly Ser Ile Asn Gln	
150 155 160 165	
AGG GAC TTT GCC CAG ATG AAG GAC TTT GTC AAA GCT TTG ATG GGA GAG	582
Arg Asp Phe Ala Gln Met Lys Asp Phe Val Lys Ala Leu Met Gly Glu	
170 175 180	
TTT GCG AGC ACC AGC ACC TTG TTC TCC CTG ATG CAA TAC TCG AAC ATC	630
Phe Ala Ser Thr Ser Thr Leu Phe Ser Leu Met Gln Tyr Ser Asn Ile	
185 190 195	
CTG AAG ACC CAT TTT ACC TTC ACT GAA TTC AAG AAC ATC CTG GAC CCT	678
Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys Asn Ile Leu Asp Pro	
200 205 210	
CAG AGC CTG GTG GAT CCC ATT GTC CAG CTG CAA GGC CTG ACC TAC ACA	726
Gln Ser Leu Val Asp Pro Ile Val Gln Leu Gln Gly Leu Thr Tyr Thr	
215 220 225	

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GCC	ACA	GGC	ATC	CGG	ACA	GTG	ATG	GAA	GAG	CTA	TTT	CAT	AGC	AAG	AAT	774
Ala	Thr	Gly	Ile	Arg	Thr	Val	Met	Glu	Glu	Leu	Phe	His	Ser	Lys	Asn	
230					235					240					245	
GGG	TCC	CGT	AAA	AGT	GCC	AAG	AAG	ATC	CTC	CTT	GTC	ATC	ACA	GAT	GGG	822
Gly	Ser	Arg	Lys	Ser	Ala	Lys	Lys	Ile	Leu	Leu	Val	Ile	Thr	Asp	Gly	
			250						255					260		
CAG	AAA	TAC	AGA	GAC	CCC	CTG	GAG	TAT	AGT	GAT	GTC	ATT	CCC	GCC	GCA	870
Gln	Lys	Tyr	Arg	Asp	Pro	Leu	Glu	Tyr	Ser	Asp	Val	Ile	Pro	Ala	Ala	
			265					270					275			
GAC	AAA	GCT	GGC	ATC	ATT	CGT	TAT	GCT	ATT	GGG	GTG	GGA	GAT	GCC	TTC	918
Asp	Lys	Ala	Gly	Ile	Ile	Arg	Tyr	Ala	Ile	Gly	Val	Gly	Asp	Ala	Phe	
		280					285					290				
CAG	GAG	CCC	ACT	GCC	CTG	AAG	GAG	CTG	AAC	ACC	ATT	GGC	TCA	GCT	CCC	966
Gln	Glu	Pro	Thr	Ala	Leu	Lys	Glu	Leu	Asn	Thr	Ile	Gly	Ser	Ala	Pro	
	295					300					305					
CCA	CAG	GAC	CAC	GTG	TTC	AAG	GTA	GGC	AAC	TTT	GCA	GCA	CTT	CGC	AGC	1014
Pro	Gln	Asp	His	Val	Phe	Lys	Val	Gly	Asn	Phe	Ala	Ala	Leu	Arg	Ser	
310					315					320					325	
ATC	CAG	AGG	CAA	CTT	CAG	GAG	AAA	ATC	TTC	GCC	ATT	GAG	GGA	ACT	CAA	1062
Ile	Gln	Arg	Gln	Leu	Gln	Glu	Lys	Ile	Phe	Ala	Ile	Glu	Gly	Thr	Gln	
				330					335					340		
TCA	AGG	TCA	AGT	AGT	TCC	TTT	CAG	CAC	GAG	ATG	TCA	CAA	GAA	GGT	TTC	1110
Ser	Arg	Ser	Ser	Ser	Ser	Phe	Gln	His	Glu	Met	Ser	Gln	Glu	Gly	Phe	
			345					350					355			
AGT	TCA	GCT	CTC	ACA	TGG	GAT	GGA	CCC	GTT	CTG	GGG	GCC	GTG	GGA	AGC	1158
Ser	Ser	Ala	Leu	Thr	Ser	Asp	Gly	Pro	Val	Leu	Gly	Ala	Val	Gly	Ser	
		360				365						370				
TTC	AGC	TGG	TCC	GGA	GGT	GCC	TTC	TTA	TAT	CCC	CCA	AAT	ACG	AGA	CCC	1206
Phe	Ser	Trp	Ser	Gly	Gly	Ala	Phe	Leu	Tyr	Pro	Pro	Asn	Thr	Arg	Pro	
	375					380					385					
ACC	TTT	ATC	AAC	ATG	TCT	CAG	GAG	AAT	GTG	GAC	ATG	AGA	GAC	TCC	TAC	1254
Thr	Phe	Ile	Asn	Met	Ser	Gln	Glu	Asn	Val	Asp	Met	Arg	Asp	Ser	Tyr	
390					395					400					405	
CTG	GGT	TAC	TCC	ACC	GCA	GTG	GCC	TTT	TGG	AAG	GGG	GTT	CAC	AGC	CTG	1302
Leu	Gly	Tyr	Ser	Thr	Ala	Val	Ala	Phe	Trp	Lys	Gly	Val	His	Ser	Leu	
				410					415					420		
ATC	CTG	GGG	GCC	CCG	CGT	CAC	CAG	CAC	ACG	GGG	AAG	GTT	GTC	ATC	TTT	1350
Ile	Leu	Gly	Ala	Pro	Arg	His	Gln	His	Thr	Gly	Lys	Val	Val	Ile	Phe	
			425					430					435			
ACC	CAG	GAA	GCC	AGG	CAT	TGG	AGG	CCC	AAG	TCT	GAA	GTC	AGA	GGG	ACA	1398
Thr	Gln	Glu	Ala	Arg	His	Trp	Arg	Pro	Lys	Ser	Glu	Val	Arg	Gly	Thr	
		440					445					450				
CAG	ATC	GGC	TCC	TAC	TTC	GGG	GCC	TCT	CTC	TGT	TCT	GTG	GAC	GTG	GAT	1446
Gln	Ile	Gly	Ser	Tyr	Phe	Gly	Ala	Ser	Leu	Cys	Ser	Val	Asp	Val	Asp	
	455					460					465					

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AGA GAT GGC AGC ACY GAC CTG GTC CTG ATC GGA GCC CCC CAT TAC TAT Arg Asp Gly Ser Xaa Asp Leu Val Leu Ile Gly Ala Pro His Tyr Tyr 470 475 480 485	1494
GAG CAG ACC CGA GGG GGG CAG GTC TCA GTG TTC CCC GTG CCC GGT GTG Glu Gln Thr Arg Gly Gln Val Ser Val Phe Pro Val Pro Gly Val 490 495 500	1542
AGG GGC AGG TGG CAG TGT GAG GCC ACC CTC CAC GGG GAG CAG GGC CAT Arg Gly Arg Trp Gln Cys Glu Ala Thr Leu His Gly Glu Gln Gly His 505 510 515	1590
CCT TGG GGC CGC TTT GGG GTG GCT CTG ACA GTG CTG GGG GAC GTA AAC Pro Trp Gly Arg Phe Gly Val Ala Leu Thr Val Leu Gly Asp Val Asn 520 525 530	1638
GGG GAC AAT CTG GCA GAC GTG GCT ATT GGT GCC CCT GGA GAG GAG GAG Gly Asp Asn Leu Ala Asp Val Ala Ile Gly Ala Pro Gly Glu Glu Glu 535 540 545	1686
AGC AGA GGT GCT GTC TAC ATA TTT CAT GGA GCC TCG AGA CTG GAG ATC Ser Arg Gly Ala Val Tyr Ile Phe His Gly Ala Ser Arg Leu Glu Ile 550 555 560 565	1734
ATG CCC TCA CCC AGC CAG CGG GTC ACT GGC TCC CAG CTC TCC CTG AGA Met Pro Ser Pro Ser Gln Arg Val Thr Gly Ser Gln Leu Ser Leu Arg 570 575 580	1782
CTG CAG TAT TTT GGG CAG TCA TTG AGT GGG GGT CAG GAC CTT ACA CAG Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly Gln Asp Leu Thr Gln 585 590 595	1830
GAT GGC CTG GTG GAC CTG GCC GTG GGA GCC CAG GGG CAC GTA CTG CTG Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln Gly His Val Leu Leu 600 605 610	1878
CTC AGG AGT CTG CCT CTG CTG AAA GTG GAG CTC TCC ATA AGA TTC GCC Leu Arg Ser Leu Pro Leu Leu Lys Val Glu Leu Ser Ile Arg Phe Ala 615 620 625	1926
CCC ATG GAG GTG GCA AAG GCT GTG TAC CAG TGC TGG GAA AGG ACT CCC Pro Met Glu Val Ala Lys Ala Val Tyr Gln Cys Trp Glu Arg Thr Pro 630 635 640 645	1974
ACT GTC CTC GAA GCT GGA GAG GCC ACT GTC TGT CTC ACT GTC CAC AAA Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys Leu Thr Val His Lys 650 655 660	2022
GGC TCA CCT GAC CTG TTA GGT AAT GTC CAA GGC TCT GTC AGG TAT GAT Gly Ser Pro Asp Leu Leu Gly Asn Val Gln Gly Ser Val Arg Tyr Asp 665 670 675	2070
CTG GCG TTA GAT CCG GCC CGC CTG ATT TCT CGT GCC ATT TTT GAT GAG Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg Ala Ile Phe Asp Glu 680 685 690	2118
ACT AAG AAC TGC ACT TTG ACG GGA AGG AAG ACT CTG GGG CTT GGT GAT Thr Lys Asn Cys Thr Leu Thr Gly Arg Lys Thr Leu Gly Leu Gly Asp 695 700 705	2166



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CAC TGC GAA ACA GTG AAG CTG CTT TTG CCG GAC TGT GTG GAA GAT GCA His Cys Glu Thr Val Lys Leu Leu Leu Pro Asp Cys Val Glu Asp Ala 710 715 720 725	2214
GTG AGC CCT ATC ATC CTG CGC CTC AAC TTT TCC CTG GTG AGA GAC TCT Val Ser Pro Ile Ile Leu Arg Leu Asn Phe Ser Leu Val Arg Asp Ser 730 735 740	2262
GCT TCA CCC AGG AAC CTG CAT CCT GTG CTG GCT GTG GGC TCA CAA GAC Ala Ser Pro Arg Asn Leu His Pro Val Leu Ala Val Gly Ser Gln Asp 745 750 755	2310
CAC ATA ACT GCT TCT CTG CCG TTT GAG AAG AAC TGT AAG CAA GAA CTC His Ile Thr Ala Ser Leu Pro Phe Glu Lys Asn Cys Lys Gln Glu Leu 760 765 770	2358
CTG TGT GAG GGG GAC CTG GGC ATC AGC TTT AAC TTC TCA GGC CTG CAG Leu Cys Glu Gly Asp Leu Gly Ile Ser Phe Asn Phe Ser Gly Leu Gln 775 780 785	2406
GTC TTG GTG GTG GGA GGC TCC CCA GAG CTC ACT GTG ACA GTC ACT GTG Val Leu Val Val Gly Gly Ser Pro Glu Leu Thr Val Thr Val Thr Val 790 795 800 805	2454
TGG AAT GAG GGT GAG GAC AGC TAT GGA ACT TTA GTC AAG TTC TAC TAC Trp Asn Glu Gly Glu Asp Ser Tyr Gly Thr Leu Val Lys Phe Tyr Tyr 810 815 820	2502
CCA GCA GGG CTA TCT TAC CGA CCG GTA ACA GGG ACT CAG CAA CCT CAT Pro Ala Gly Leu Ser Tyr Arg Arg Val Thr Gly Thr Gln Gln Pro His 825 830 835	2550
CAG TAC CCA CTA CGC TTG GCC TGT GAG GCT GAG CCC GCT GCC CAG GAG Gln Tyr Pro Leu Arg Leu Ala Cys Glu Ala Glu Pro Ala Ala Gln Glu 840 845 850	2598
GAC CTG AGG AGC AGC AGC TGT AGC ATT AAT CAC CCC ATC TTC CGA GAA Asp Leu Arg Ser Ser Ser Cys Ser Ile Asn His Pro Ile Phe Arg Glu 855 860 865	2646
GGT GCA AAG ACC ACC TTC ATG ATC ACA TTC GAT GTC TCC TAC AAG GCC Gly Ala Lys Thr Thr Phe Met Ile Thr Phe Asp Val Ser Tyr Lys Ala 870 875 880 885	2694
TTC CTA GGA GAC AGG TTG CTT CTG AGG GCC AAA GCC AGC AGT GAG AAT Phe Leu Gly Asp Arg Leu Leu Leu Arg Ala Lys Ala Ser Ser Glu Asn 890 895 900	2742
AAT AAG CCT GAT ACC AAC AAG ACT GCC TTC CAG CTG GAG CTC CCA GTG Asn Lys Pro Asp Thr Asn Lys Thr Ala Phe Gln Leu Glu Leu Pro Val 905 910 915	2790
AAG TAC ACC GTC TAT ACC CTG ATC AGT AGG CAA GAA GAT TCC ACC AAC Lys Tyr Thr Val Tyr Thr Leu Ile Ser Arg Gln Glu Asp Ser Thr Asn 920 925 930	2838
CAT GTC AAC TTT TCA TCT TCC CAC GGG GGG AGA AGG CAA GAA GCC GCA His Val Asn Phe Ser Ser Ser His Gly Gly Arg Arg Gln Glu Ala Ala 935 940 945	2886

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CAT CGC TAT CGT GTG AAT AAC CTG AGT CCA CTG AAG CTG GCC GTC AGA His Arg Tyr Arg Val Asn Asn Leu Ser Pro Leu Lys Leu Ala Val Arg 950 955 960 965	2934
GTT AAC TTC TGG GTC CCT GTC CTT CTG AAC GGT GTG GCT GTG TGG GAC Val Asn Phe Trp Val Pro Val Leu Leu Asn Gly Val Ala Val Trp Asp 970 975 980	2982
GTG ACT CTG AGC AGC CCA GCA CAG GGT GTC TCC TGC GTG TCC CAG ATG Val Thr Leu Ser Ser Pro Ala Gln Gly Val Ser Cys Val Ser Gln Met 985 990 995	3030
AAA CCT CCT CAG AAT CCC GAC TTT CTG ACC CAG ATT CAG AGA CGT TCT Lys Pro Pro Gln Asn Pro Asp Phe Leu Thr Gln Ile Gln Arg Arg Ser 1000 1005 1010	3078
GTG CTG GAC TGC TCC ATT GCT GAC TGC CTG CAC TTC CGC TGT GAC ATC Val Leu Asp Cys Ser Ile Ala Asp Cys Leu His Phe Arg Cys Asp Ile 1015 1020 1025	3126
CCC TCC TTG GAC ATC CAG GAT GAA CTT GAC TTC ATT CTG AGG GGC AAC Pro Ser Leu Asp Ile Gln Asp Glu Leu Asp Phe Ile Leu Arg Gly Asn 1030 1035 1040 1045	3174
CTC AGC TTC GGC TGG GTC AGT CAG ACA TTG CAG GAA AAG GTG TTG CTT Leu Ser Phe Gly Trp Val Ser Gln Thr Leu Gln Glu Lys Val Leu Leu 1050 1055 1060	3222
GTG AGT GAG GCT GAA ATC ACT TTC GAC ACA TCT GTG TAC TCC CAG CTG Val Ser Glu Ala Glu Ile Thr Phe Asp Thr Ser Val Tyr Ser Gln Leu 1065 1070 1075	3270
CCA GGA CAG GAG GCA TTT CTG AGA GCC CAG GTG GAG ACA ACG TTA GAA Pro Gly Gln Glu Ala Phe Leu Arg Ala Gln Val Glu Thr Thr Leu Glu 1080 1085 1090	3318
GAA TAC GTG GTC TAT GAG CCC ATC TTC CTC GTG GCG GGC AGC TCG GTG Glu Tyr Val Val Tyr Glu Pro Ile Phe Leu Val Ala Gly Ser Ser Val 1095 1100 1105	3366
GGA GGT CTG CTG TTA CTG GCT CTC ATC ACA GTG GTA CTG TAC AAG CTT Gly Gly Leu Leu Leu Leu Ala Leu Ile Thr Val Val Leu Tyr Lys Leu 1110 1115 1120 1125	3414
GGC TTC TYC AAA CGT CAG TAC AAA GAA ATG CTG GAC GGC AAG GCT GCA Gly Phe Xaa Lys Arg Gln Tyr Lys Glu Met Leu Asp Gly Lys Ala Ala 1130 1135 1140	3462
GAT CCT GTC ACA GCC GGC CAG GCA GAT TTC GGC TGT GAG ACT CCT CCA Asp Pro Val Thr Ala Gly Gln Ala Asp Phe Gly Cys Glu Thr Pro Pro 1145 1150 1155	3510
TAT CTC GTG AGC TAGGAATCCA CTCTCTGCC TATCTCTGCA ATGAAGATTG Tyr Leu Val Ser 1160	3562
GTCTCTGCCTA TGAGTCTACT GGCATGGGAA CGAGT	3597

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## (2) INFORMATION FOR SEQ ID NO:55:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1161 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```

Met Ala Gly Gly Val Val Ile Leu Leu Cys Gly Trp Val Leu Ala Ser
 1           5           10           15
Cys His Gly Ser Asn Leu Asp Val Glu Glu Pro Ile Val Phe Arg Glu
          20           25           30
Asp Ala Ala Ser Phe Gly Gln Thr Val Val Gln Phe Gly Gly Ser Arg
          35           40           45
Leu Val Val Gly Ala Pro Leu Glu Ala Val Ala Val Asn Gln Thr Gly
50           55           60
Arg Leu Tyr Asp Cys Ala Pro Ala Thr Gly Met Cys Gln Pro Ile Val
65           70           75           80
Leu Arg Ser Pro Leu Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu
          85           90           95
Val Thr Ala Thr Asn Asn Ala Gln Leu Leu Ala Cys Gly Pro Thr Ala
          100          105          110
Gln Arg Ala Cys Val Lys Asn Met Tyr Ala Lys Gly Ser Cys Leu Leu
          115          120          125
Leu Gly Ser Ser Leu Gln Phe Ile Gln Ala Val Pro Ala Ser Met Pro
          130          135          140
Glu Cys Pro Arg Gln Glu Met Asp Ile Ala Phe Leu Ile Asp Gly Ser
145          150          155          160
Gly Ser Ile Asn Gln Arg Asp Phe Ala Gln Met Lys Asp Phe Val Lys
          165          170          175
Ala Leu Met Gly Glu Phe Ala Ser Thr Ser Thr Leu Phe Ser Leu Met
          180          185          190
Gln Tyr Ser Asn Ile Leu Lys Thr His Phe Thr Phe Thr Glu Phe Lys
          195          200          205
Asn Ile Leu Asp Pro Gln Ser Leu Val Asp Pro Ile Val Gln Leu Gln
          210          215          220
Gly Leu Thr Tyr Thr Ala Thr Gly Ile Arg Thr Val Met Glu Glu Leu
225          230          235          240
Phe His Ser Lys Asn Gly Ser Arg Lys Ser Ala Lys Lys Ile Leu Leu
          245          250          255
Val Ile Thr Asp Gly Gln Lys Tyr Arg Asp Pro Leu Glu Tyr Ser Asp
          260          265          270

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Val Ile Pro Ala Ala Asp Lys Ala Gly Ile Ile Arg Tyr Ala Ile Gly  
 275 280 285  
 Val Gly Asp Ala Phe Gln Glu Pro Thr Ala Leu Lys Glu Leu Asn Thr  
 290 295 300  
 Ile Gly Ser Ala Pro Pro Gln Asp His Val Phe Lys Val Gly Asn Phe  
 305 310 315 320  
 Ala Ala Leu Arg Ser Ile Gln Arg Gln Leu Gln Glu Lys Ile Phe Ala  
 325 330 335  
 Ile Glu Gly Thr Gln Ser Arg Ser Ser Ser Phe Gln His Glu Met  
 340 345 350  
 Ser Gln Glu Gly Phe Ser Ser Ala Leu Thr Ser Asp Gly Pro Val Leu  
 355 360 365  
 Gly Ala Val Gly Ser Phe Ser Trp Ser Gly Gly Ala Phe Leu Tyr Pro  
 370 375 380  
 Pro Asn Thr Arg Pro Thr Phe Ile Asn Met Ser Gln Glu Asn Val Asp  
 385 390 395 400  
 Met Arg Asp Ser Tyr Leu Gly Tyr Ser Thr Ala Val Ala Phe Trp Lys  
 405 410 415  
 Gly Val His Ser Leu Ile Leu Gly Ala Pro Arg His Gln His Thr Gly  
 420 425 430  
 Lys Val Val Ile Phe Thr Gln Glu Ala Arg His Trp Arg Pro Lys Ser  
 435 440 445  
 Glu Val Arg Gly Thr Gln Ile Gly Ser Tyr Phe Gly Ala Ser Leu Cys  
 450 455 460  
 Ser Val Asp Val Asp Arg Asp Gly Ser Xaa Asp Leu Val Leu Ile Gly  
 465 470 475 480  
 Ala Pro His Tyr Tyr Glu Gln Thr Arg Gly Gly Gln Val Ser Val Phe  
 485 490 495  
 Pro Val Pro Gly Val Arg Gly Arg Trp Gln Cys Glu Ala Thr Leu His  
 500 505 510  
 Gly Glu Gln Gly His Pro Trp Gly Arg Phe Gly Val Ala Leu Thr Val  
 515 520 525  
 Leu Gly Asp Val Asn Gly Asp Asn Leu Ala Asp Val Ala Ile Gly Ala  
 530 535 540  
 Pro Gly Glu Glu Glu Ser Arg Gly Ala Val Tyr Ile Phe His Gly Ala  
 545 550 555 560  
 Ser Arg Leu Glu Ile Met Pro Ser Pro Ser Gln Arg Val Thr Gly Ser  
 565 570 575  
 Gln Leu Ser Leu Arg Leu Gln Tyr Phe Gly Gln Ser Leu Ser Gly Gly  
 580 585 590

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Gln Asp Leu Thr Gln Asp Gly Leu Val Asp Leu Ala Val Gly Ala Gln  
 595 600 605  
 Gly His Val Leu Leu Leu Arg Ser Leu Pro Leu Leu Lys Val Glu Leu  
 610 615 620  
 Ser Ile Arg Phe Ala Pro Met Glu Val Ala Lys Ala Val Tyr Gln Cys  
 625 630 635 640  
 Trp Glu Arg Thr Pro Thr Val Leu Glu Ala Gly Glu Ala Thr Val Cys  
 645 650 655  
 Leu Thr Val His Lys Gly Ser Pro Asp Leu Leu Gly Asn Val Gln Gly  
 660 665 670  
 Ser Val Arg Tyr Asp Leu Ala Leu Asp Pro Gly Arg Leu Ile Ser Arg  
 675 680 685  
 Ala Ile Phe Asp Glu Thr Lys Asn Cys Thr Leu Thr Gly Arg Lys Thr  
 690 695 700  
 Leu Gly Leu Gly Asp His Cys Glu Thr Val Lys Leu Leu Leu Pro Asp  
 705 710 715 720  
 Cys Val Glu Asp Ala Val Ser Pro Ile Ile Leu Arg Leu Asn Phe Ser  
 725 730 735  
 Leu Val Arg Asp Ser Ala Ser Pro Arg Asn Leu His Pro Val Leu Ala  
 740 745 750  
 Val Gly Ser Gln Asp His Ile Thr Ala Ser Leu Pro Phe Glu Lys Asn  
 755 760 765  
 Cys Lys Gln Glu Leu Leu Cys Glu Gly Asp Leu Gly Ile Ser Phe Asn  
 770 775 780  
 Phe Ser Gly Leu Gln Val Leu Val Val Gly Gly Ser Pro Glu Leu Thr  
 785 790 795 800  
 Val Thr Val Thr Val Trp Asn Glu Gly Glu Asp Ser Tyr Gly Thr Leu  
 805 810 815  
 Val Lys Phe Tyr Tyr Pro Ala Gly Leu Ser Tyr Arg Arg Val Thr Gly  
 820 825 830  
 Thr Gln Gln Pro His Gln Tyr Pro Leu Arg Leu Ala Cys Glu Ala Glu  
 835 840 845  
 Pro Ala Ala Gln Glu Asp Leu Arg Ser Ser Ser Cys Ser Ile Asn His  
 850 855 860  
 Pro Ile Phe Arg Glu Gly Ala Lys Thr Thr Phe Met Ile Thr Phe Asp  
 865 870 875 880  
 Val Ser Tyr Lys Ala Phe Leu Gly Asp Arg Leu Leu Leu Arg Ala Lys  
 885 890 895  
 Ala Ser Ser Glu Asn Asn Lys Pro Asp Thr Asn Lys Thr Ala Phe Gln  
 900 905 910

Leu	Glu	Leu	Pro	Val	Lys	Tyr	Thr	Val	Tyr	Thr	Leu	Ile	Ser	Arg	Gln
	915							920							
Glu	Asp	Ser	Thr	Asn	His	Val	Asn	Phe	Ser	Ser	Ser	His	Gly	Gly	Arg
	930					935					940				
Arg	Gln	Glu	Ala	Ala	His	Arg	Tyr	Arg	Val	Asn	Asn	Leu	Ser	Pro	Leu
945					950					955					960
Lys	Leu	Ala	Val	Arg	Val	Asn	Phe	Trp	Val	Pro	Val	Leu	Leu	Asn	Gly
				965					970					975	
Val	Ala	Val	Trp	Asp	Val	Thr	Leu	Ser	Ser	Pro	Ala	Gln	Gly	Val	Ser
			980					985					990		
Cys	Val	Ser	Gln	Met	Lys	Pro	Pro	Gln	Asn	Pro	Asp	Phe	Leu	Thr	Gln
	995						1000					1005			
Ile	Gln	Arg	Arg	Ser	Val	Leu	Asp	Cys	Ser	Ile	Ala	Asp	Cys	Leu	His
1010						1015					1020				
Phe	Arg	Cys	Asp	Ile	Pro	Ser	Leu	Asp	Ile	Gln	Asp	Glu	Leu	Asp	Phe
1025					1030					1035					1040
Ile	Leu	Arg	Gly	Asn	Leu	Ser	Phe	Gly	Trp	Val	Ser	Gln	Thr	Leu	Gln
				1045					1050					1055	
Glu	Lys	Val	Leu	Leu	Val	Ser	Glu	Ala	Glu	Ile	Thr	Phe	Asp	Thr	Ser
			1060					1065					1070		
Val	Tyr	Ser	Gln	Leu	Pro	Gly	Gln	Glu	Ala	Phe	Leu	Arg	Ala	Gln	Val
	1075						1080					1085			
Glu	Thr	Thr	Leu	Glu	Glu	Tyr	Val	Val	Tyr	Glu	Pro	Ile	Phe	Leu	Val
	1090					1095					1100				
Ala	Gly	Ser	Ser	Val	Gly	Gly	Leu	Leu	Leu	Leu	Ala	Leu	Ile	Thr	Val
1105					1110						1115				1120
Val	Leu	Tyr	Lys	Leu	Gly	Xaa	Xaa	Lys	Arg	Gln	Tyr	Lys	Glu	Met	Leu
				1125					1130					1135	
Asp	Gly	Lys	Ala	Ala	Asp	Pro	Val	Thr	Xaa	Gly	Gln	Ala	Asp	Phe	Gly
			1140					1145					1150		
Cys	Glu	Thr	Pro	Pro	Tyr	Leu	Val	Ser							
	1155							1160							

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

BNSDOCID: &lt;WO 9517412A1 | &gt;

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CCTGTCATGG GTCTAACCTG

20

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

AGGTTAGACC CATGACAGG

19

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGCCTTGCAG CTGGACAATG

20

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CCAAAGCTGG CTGCATCCTC TC

22

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCGCCTGCCA CTGGCGTGTG C

21

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CCCAGATGAA GGACTTCGTC AA

22

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GCTGGGATCA TTCGCTATGC

20

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CAATGGATGG ACCAGTTCTG G

21

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear



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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CAGATCGGCT CCTACTTTGG

20

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CATGGAGCCT CGAGACAGG

19

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CCACTGTCCT CGAAGCTGGA G

21

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CTTCGTCCTG TGCTGGCTGT GGGCTC

26

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CGCCTGGCAT GTGAGGCTGA G

21

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CCGTGATCAG TAGGCAGGAA G

21

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GTCACAGAGG GAACCTCC

18

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GCTCCTGAGT GAGGCTGAAA TCA

23

(2) INFORMATION FOR SEQ ID NO:72:

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- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GAGATGCTGG ATCTACCATC TGC

23

(2) INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CTGAGCTGGG AGATTTTAT GG

22

(2) INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GTGGATCAGC ACTGAAATCT G

21

(2) INFORMATION FOR SEQ ID NO:75:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CGTTTGAAGA AGCCAAGCTT G

21

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## (2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CACAGCGGAG GTGCAGGCAG

20

## (2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CTCACTGCTT GCGCTGGC

18

## (2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CGGTAAGATA GCTCTGCTGG

20

## (2) INFORMATION FOR SEQ ID NO:79:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

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GAGCCACAG CCAGCACAGG

20

## (2) INFORMATION FOR SEQ ID NO:80:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GATCCAACGC CAGATCATAC C

21

## (2) INFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CACGGCCAGG TCCACCAGGC

20

## (2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CACGTCCCCT AGCACTGTCA G

21

## (2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TTGACGAAGT CCTTCATCTG GG

22

(2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GAACTGCAAG CTGGAGCCCA G

21

(2) INFORMATION FOR SEQ ID NO:85:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

CTGGATGCTG CGAAGTGCTA C

21

(2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GCCTTGGAGC TGGACGATGG C

21

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GTAAGATCTC CAGAGTGTCC AAGACAAGAG ATG

33

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CTTCTCGAGT GTGAGAGCTG AACTGAAACC TTC

33

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

CGCTGTGACG TCAGAGTTGA GTCCAAATAT GG

32

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GGTGACACTA TAGAATAGGG C

21

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

AAGCAGGAGCTCCTGTGT

18

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 852 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 61..852

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

TGATCTCCCT CCAGGCCACT GTTCCCTCTC CACTTCCCCT CACCGCTGCA CTGCTCAGAG	60
ATG GCC CTT GGG GCT GTG GTC CTC CTT GGG GTC CTG GCT TCT TAC CAC	108
Met Ala Leu Gly Ala Val Val Leu Leu Gly Val Leu Ala Ser Tyr His	
1 5 10 15	
GGA TTC AAC TTG GAC GTG ATG AGC GGT GAT CTT CCA GCA AGA CGC AGC	156
Gly Phe Asn Leu Asp Val Met Ser Gly Asp Leu Pro Gly Arg Arg Ser	
20 25 30	
GGG CTT CGG GCA GAG CGT GAT GCA GTT TGG GGA TCT CGA CTC GTG GTG	204
Gly Leu Arg Ala Glu Arg Asp Ala Val Trp Gly Ser Arg Leu Val Val	
35 40 45	
GGA GCC CCC CTG GCG GTG GTG TCG GCC AAC CAC ACA GGA CGG CTG TAC	252
Gly Ala Pro Leu Ala Val Val Ser Ala Asn His Thr Gly Arg Leu Tyr	
50 55 60	
GAG TGT GCG CCT GCC TCC GGC ACC TGC ACG CCC ATT TTC CCA TTC ATG	300
Glu Cys Ala Pro Ala Ser Gly Thr Cys Thr Pro Ile Phe Pro Phe Met	
65 70 75 80	
CCC CCC GAA GCC GTG AAC ATG TCC CTG GGC CTG TCC CTG GCA GCC TCC	348
Pro Pro Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu Ala Ala Ser	
85 90 95	
CCC AAC CAT TCC CAG CTG CTG GCT TGT GGC CCG ACC GTG CAT AGA GCC	396
Pro Asn His Ser Gln Leu Leu Ala Cys Gly Pro Thr Val His Arg Ala	
100 105 110	



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TGC GGG GAG GAC GTG TAC GCC CAG GGT TTC TGT GTG CTG CTG GAT GCC Cys Gly Glu Asp Val Tyr Ala Gln Gly Phe Cys Val Leu Leu Asp Ala 115 120 125	444
CAC GCA CAG CCC ATC GGG ACT GTG CCA GCT GCC CTG CCC GAG TGC CCA His Ala Gln Pro Ile Gly Thr Val Pro Ala Ala Leu Pro Glu Cys Pro 130 135 140	492
GAT CAA GAG ATG GAC ATT GTC TTC CTG ATT GAC GGC TCT GGC AGC ATT Asp Gln Glu Met Asp Ile Val Phe Leu Ile Asp Gly Ser Gly Ser Ile 145 150 155 160	540
AGC TCA AAT GAC TTC CGC AAG ATG AAG GAC TTT GTC AGA GCT GTG ATG Ser Ser Asn Asp Phe Arg Lys Met Lys Asp Phe Val Arg Ala Val Met 165 170 175	588
GAC CAG TTC AAG GAC ACC AAC ACC CAG TTC TCG CTG ATG CAG TAC TCC Asp Gln Phe Lys Asp Thr Asn Thr Gln Phe Ser Leu Met Gln Tyr Ser 180 185 190	636
AAT GTG CTG GTG ACA CAT TTC ACC TTC AGC AGC TTC CGG AAC AGC TCC Asn Val Leu Val Thr His Phe Thr Phe Ser Ser Phe Arg Asn Ser Ser 195 200 205	684
AAT CCT CAG GGC CTA GTG GAG CCC ATT GTG CAG CTG ACA GGC CTC ACG Asn Pro Gln Gly Leu Val Glu Pro Ile Val Gln Leu Thr Gly Leu Thr 210 215 220	732
TTC ACG GCC ACA GGG ATC CTG AAA GTG GTG ACA GAG CTG TTT CAA ACC Phe Thr Ala Thr Gly Ile Leu Lys Val Val Thr Glu Leu Phe Gln Thr 225 230 235 240	780
AAG AAC GGG GCC CGC GAA AGT GCC AAG AAG ATC CTC ATC GTC ATC ACA Lys Asn Gly Ala Arg Glu Ser Ala Lys Lys Ile Leu Ile Val Ile Thr 245 250 255	828
GAT GGG CAG AAG TAC AAA GCG GCA Asp Gly Gln Lys Tyr Lys Ala Ala 260	852

## (2) INFORMATION FOR SEQ ID NO:93:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 264 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Met	Ala	Leu	Gly	Ala	Val	Val	Leu	Leu	Gly	Val	Leu	Ala	Ser	Tyr	His
1				5					10					15	
Gly	Phe	Asn	Leu	Asp	Val	Met	Ser	Gly	Asp	Leu	Pro	Gly	Arg	Arg	Ser
		20						25					30		
Gly	Leu	Arg	Ala	Glu	Arg	Asp	Ala	Val	Trp	Gly	Ser	Arg	Leu	Val	Val
		35					40					45			

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Gly Ala Pro Leu Ala Val Val Ser Ala Asn His Thr Gly Arg Leu Tyr  
 50 55 60  
 Glu Cys Ala Pro Ala Ser Gly Thr Cys Thr Pro Ile Phe Pro Phe Met  
 65 70 75 80  
 Pro Pro Glu Ala Val Asn Met Ser Leu Gly Leu Ser Leu Ala Ala Ser  
 85 90 95  
 Pro Asn His Ser Gln Leu Leu Ala Cys Gly Pro Thr Val His Arg Ala  
 100 105 110  
 Cys Gly Glu Asp Val Tyr Ala Gln Gly Phe Cys Val Leu Leu Asp Ala  
 115 120 125  
 His Ala Gln Pro Ile Gly Thr Val Pro Ala Ala Leu Pro Glu Cys Pro  
 130 135 140  
 Asp Gln Glu Met Asp Ile Val Phe Leu Ile Asp Gly Ser Gly Ser Ile  
 145 150 155 160  
 Ser Ser Asn Asp Phe Arg Lys Met Lys Asp Phe Val Arg Ala Val Met  
 165 170 175  
 Asp Gln Phe Lys Asp Thr Asn Thr Gln Phe Ser Leu Met Gln Tyr Ser  
 180 185 190  
 Asn Val Leu Val Thr His Phe Thr Phe Ser Ser Phe Arg Asn Ser Ser  
 195 200 205  
 Asn Pro Gln Gly Leu Val Glu Pro Ile Val Gln Leu Thr Gly Leu Thr  
 210 215 220  
 Phe Thr Ala Thr Gly Ile Leu Lys Val Val Thr Glu Leu Phe Gln Thr  
 225 230 235 240  
 Lys Asn Gly Ala Arg Glu Ser Ala Lys Lys Ile Leu Ile Val Ile Thr  
 245 250 255  
 Asp Gly Gln Lys Tyr Lys Ala Ala  
 260

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WHAT IS CLAIMED IS:

1. A purified and isolated  $\alpha_d$  polynucleotide consisting essentially of human  $\alpha_d$  protein coding sequence set out in SEQ ID NO: 1.
2. The polynucleotide of claim 1 which is a DNA molecule.
3. The DNA molecule of claim 2 which is a cDNA molecule.
4. The DNA molecule of claim 2 which is a genomic DNA molecule.
5. The DNA molecule of claim 2 which is a wholly or partially chemically synthesized DNA molecule.
6. A full length purified and isolated  $\alpha_d$ -encoding polynucleotide selected from the group consisting of:
  - a) the human DNA sequence set out in SEQ ID NO: 1, and
  - b) a DNA molecule which hybridizes under stringent conditions to the noncoding strand of the protein coding portion of the DNA of a).
7. A DNA molecule encoding the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.
8. A DNA expression construct comprising a DNA molecule according to claim 2.
9. A host cell transformed with a DNA molecule according to claim 2.

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10. A method for producing an  $\alpha_d$  polypeptide comprising growing a host cell according to claim 9 in a suitable medium and isolating  $\alpha_d$  polypeptide from said host cell or the medium of its growth.

11. Purified and isolated  $\alpha_d$  polypeptide consisting essentially of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

12. A polypeptide capable of specifically binding to  $\alpha_d$ .

13. A polypeptide according to claim 12 which is an antibody.

14. An antibody according to claim 13 which is a monoclonal antibody.

15. An anti-idiotypic antibody specific for the monoclonal antibody of claim 14.

16. A hybridoma cell line producing the monoclonal antibody according to claim 14.

17. A purified and isolated  $\alpha_d$  extracellular domain polypeptide fragment comprising amino acids 17 to 1108 of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

18. A purified and isolated  $\alpha_d$  I domain polypeptide fragment comprising amino acids 145 to 355 of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

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19. A fusion protein comprising  $\alpha_d$  extracellular domain polypeptide amino acids 17 to 1108 of SEQ ID NO: 2 and human immunoglobulin constant domain sequences.

20. A purified and isolated murine polynucleotide consisting essentially of the  $\alpha$  subunit protein coding sequence set out in SEQ ID NO: 45.

21. A method for isolating a polynucleotide encoding a protein that binds to  $\alpha_d$  comprising the steps of:

a) transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain;

b) expressing in said host cells a first hybrid DNA sequence encoding a first fusion of part or all of  $\alpha_d$  and either the DNA binding domain or the activating domain of said transcription factor;

c) expressing in said host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative  $\alpha_d$  binding proteins and the DNA binding domain or activating domain of said transcription factor which is not incorporated in said first fusion;

d) detecting binding of an  $\alpha_d$  binding protein to  $\alpha_d$  in a particular host cell by detecting the production of reporter gene product in said host cell; and

e) isolating second hybrid DNA sequences encoding  $\alpha_d$  binding protein from said particular host cell.

22. A method for identifying a compound capable of reacting specifically with  $\alpha_d$  and of modulating the interaction of binding partners  $\alpha_d$  and ICAM-R comprising the steps of:

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a) immobilizing  $\alpha_d$  or a fragment thereof, or ICAM-R or a fragment thereof, on a solid support coated or impregnated with a fluorescent agent;

b) labelling the non-immobilized binding partner with a compound capable of exciting said fluorescent agent;

c) contacting said immobilized binding partner with said labelled binding partner in the presence and absence of a putative modulator compound capable of specifically reacting with  $\alpha_d$ ;

d) detecting light emission by said fluorescent agent; and

e) identifying modulating compounds as those compounds that affect the emission of light by said fluorescent agent in comparison to the emission of light by said fluorescent agent in the absence of said modulating compound.

23. A purified and isolated  $\alpha_d$  extracellular domain polypeptide fragment comprising about amino acid 127 to about amino acid 353 of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

24. A fusion protein comprising the polypeptide fragment of claim 23 and human immunoglobulin constant region sequences.

25. A purified and isolated  $\alpha_d$  extracellular domain polypeptide fragment comprising about amino acid 17 to about amino acid 603 of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

26. A fusion protein comprising the polypeptide fragment of claim 25 and human immunoglobulin constant region sequences.

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27. A purified and isolated  $\alpha_d$  extracellular domain polypeptide fragment comprising about amino acid 17 to about amino acid 1029 of the human  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 2.

28. A fusion protein comprising the polypeptide fragment of claim 27 and immunoglobulin constant region sequences.

29. A purified and isolated murine polynucleotide comprising the  $\alpha$  subunit protein coding sequence as set out in SEQ ID NO: 52.

30. A purified and isolated  $\alpha_d$  polypeptide consisting essentially of the murine  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 53.

31. A purified and isolated rat polynucleotide comprising the  $\alpha$  subunit protein coding sequence as set out in SEQ ID NO: 54.

32. A purified and isolated  $\alpha_d$  polypeptide consisting essentially of the rat  $\alpha_d$  amino acid sequence set out in SEQ ID NO: 55.

33. A purified and isolated polypeptide fragment comprising extracellular domain sequences of the polypeptide of claim 32.

34. A polypeptide capable of specifically binding to the polypeptide of claim 32.

35. A polypeptide according to claim 34 which is an antibody.

36. An antibody according to claim 35 which is a monoclonal antibody.

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37. A rodent that does not express a functional  $\alpha_d$  protein.

38. A rodent that expresses a variant  $\alpha_d$  protein.



$\alpha$ D	TF-GT--VLL	LSVLASYHGF	NLDVEEPTIF	QEDAGGFGQS	VVQFGGSRLV	47
CD11B	MA-LR--VLL	LTALTCHGF	NLDTENAMTF	OENARGFGQS	VVQLQGSRVV	47
CD11C	MTRTRAALLL	FTALATSLGF	NLDTEELTAF	RVDSAGFGDS	VVQYANSWVV	50
$\alpha$ D	VGAPLEVVA	NOTGRLYDCA	AATGMCPIP	LHIRPEAVNM	SLGLTLAAT	97
CD11B	VGAPQEIIVA	NORGSLYQCD	YSTGSCEPIR	LQVPVEAVNM	SLGLSLAATT	97
CD11C	VGAPQKIIAA	NOIGGLYQCG	YSTGACEPIG	LQVPPEAVNM	SLGLSLASTT	100
$\alpha$ D	NGSRLLACGP	TLHRVCGENS	YSKGSCLLLG	SR-WEIIQTV	PDATPECPHQ	146
CD11B	SPPQLLACGP	TVHOTCSENT	YVKGLCFLFG	SNLRQOPQKF	PEALRGCPQE	147
CD11C	SPSQLLACGP	TVHHECGRNM	YLTGLCFLLG	PT--QLTORL	PVSRQECPRQ	148
$\alpha$ D	EMDIVFLIDG	SGSIDQNDFN	OMKGFVQAVM	GQFEGTDTLF	ALMQYSNLLK	196
CD11B	DSDIAFLIDG	SGSIIPHDFR	RMKEFVSTVM	EQLKSKTLF	SLMQYSEEF	197
CD11C	EODIVFLIDG	SGSISRNFA	TMMNFYRAVI	SQFORPSTQF	SLMQFSNKFO	198
$\alpha$ D	IHFTFTQFRT	SPSQOQSLVDP	IVOLKGLTFT	ATGILTVVTQ	LFHHKNGARK	246
CD11B	IHFTTFKEFON	NPNRSLVKP	ITQLLGRTHI	ATGIRKVVRE	LFNITNGARK	247
CD11C	THFTFEEFRR	TSNPLSLLAS	VHQLQGFTYT	ATAIONVVHR	LFHASYGARR	248
$\alpha$ D	SAKKILIVIT	DGOKYKDPLE	YSDVIPQAEK	AGIIRYAIGV	GHAFOGPTAR	296
CD11B	NAFKILVVIT	DGEKFGDPLG	YEDVIPPADR	EGVIRYVIGV	GDAFRSEKSR	297
CD11C	DAIKILIVIT	DGKKEGDSLD	YKDVIPMADA	AGIIRYAIGV	GLAFONRNSW	298

FIGURE 1A

$\alpha$ D	OELNTISSAP	PODHVFKVDN	FAALGSIOKQ	LOEKIYAVEG	TOSRASSSFO	346
CD11B	OELNTIASKP	PRDHVFOVNN	FEALKTIONQ	LREKIFAIEG	TQTGSSSSFE	347
CD11C	KELNDIASKP	SOEHIFKVED	FDALKDIONQ	LKEKIFAIEG	TETISSSSFE	348
$\alpha$ D	HEMSOEGFST	ALTHDGLFLG	AVGSFSWSGG	AFLYPPNMSP	TFINMSQENV	396
CD11B	HEMSOEGFSA	AITSNGLPLS	TVGSYDHWAGG	VFLYTSKEKS	TFINMTRVDS	397
CD11C	LEMAOEGFSA	VFTPDGPVLG	AVGSFTWSGG	AFLYPPNMSP	TFINMSQENV	398
$\alpha$ D	DNRDSYLGYS	TELALWKGVQ	NLVLGAPRYQ	HTGKAVIFIQ	VSRQWRKKAE	446
CD11B	DMNDAYLGVA	AAIILNRVQ	SLVLGAPRYQ	HIGLVAFERQ	NTGMWESWAN	447
CD11C	DNRDSYLGYS	TELALWKGVQ	SLVLGAPRYQ	HIGKAVIFIQ	VSRQWRMKAE	448
$\alpha$ D	VTGTQIGSYF	GASLCSVDVD	SDGSTDLILI	GAPHYVEQTR	GGQVSVCPLP	496
CD11B	VKGTOIGAYF	GASLCSVDVD	SNGSTDLVLI	GAPHYVEQTR	GGQVSVCPLP	497
CD11C	VIGTQIGSYF	GASLCSVDVD	TDGSTDLVLI	GAPHYVEQTR	GGQVSVCPLP	498
$\alpha$ D	RGORVQWQCD	AVLRGEQGHF	WGRFGAALT	LGDVNEKLI	DVAIGAPGEE	546
CD11B	RGORARWQCD	AVLYGEQGHF	WGRFGAALT	LGDVNGDKLT	DVAIGAPGEE	547
CD11C	RGWRRW-CD	AVLYGEQGHF	WGRFGAALT	LGDVNGDKLT	DVVIAPGEE	547
$\alpha$ D	ENRGAVYLFH	GASESGISPS	HSQRIASSQL	SPRLQYFGOA	LSGGODLTOD	596
CD11B	DNRGAVYLFH	GTSCSGISPS	HSQRIAGSKL	SPRLQYFGOS	LSGGODLTMD	597
CD11C	ENRGAVYLFH	GVLGPSISPS	HSQRIAGSQL	SSRLQYFGOA	LSGGODLTOD	597

FIGURE 1B

$\alpha$ D	GLMDLAVGAR	GOVLLLRSLP	VLKVGAMRF	SPVEKAVY	RCWEEKPSAL	646
CD11B	GLVDLTVGAG	GHVLLRSQP	VLRVKAIMF	NPREVARNVF	ECNDQVVKGK	647
CD11C	GLVDLAVGAR	GOVLLRTRP	VLWVGSMQF	IPAEIPRSF	ECREQVVSQ	647
$\alpha$ D	EAGDATVCLT	IOKSSLDQL-	-GDIOSSVF	DLALDPGRLT	SRAIFNETKN	694
CD11B	EAGEVRVCLH	VOKSTRDRLR	EGQIQSVVTY	DLALDSGRPH	SRAVFNETKN	697
CD11C	TLVQSNICLY	IDKRSKNLLG	SRDLQSSVTL	DLALAPGRLS	PRAIFQETKN	697
$\alpha$ D	PTLTTRKTLG	LGIHCETLKL	LLPDCVEDVV	SPIILHLNFS	LVREPIPSQ	744
CD11B	STRRTQVLG	LTQTCETLKL	QLPNCIEDPV	SPIVLRNFS	LVGTPLSAFG	747
CD11C	RSLSRVRVLG	LKAHCENFNL	LLPSCVEDSV	IPIILRENET	LVGKPLLAFR	747
$\alpha$ D	NLRPVLAVGS	ODLFTASLPF	EKNCGQDGLC	EGDLGVTLSF	SGLOTTLTVGS	794
CD11B	NLRPVLAEAD	ORLFTALFPF	EKNCGNDNIC	ODDLSITFSF	MSLDCLVVG	797
CD11C	NLRPMLAALA	QRYFTASLPF	EKNCGADHIC	QDNLGISFSF	PGLKSLLVGS	797
$\alpha$ D	SLELNIVTV	WNAGEDSYGT	VVSLYYPAGL	SHRRVSGAQK	OPHQSALRLA	844
CD11B	PREFNVTIV	RNDGEDSYRT	QVTFFPLDL	SYRKVSTLQ	QSRQSRWRLA	847
CD11C	NLELNAEVMV	WNGEDSYGT	TITFHPAGL	SYRYVAEGOK	QGQLRSLHLT	847
$\alpha$ D	CETVPTD--	EGLRSSRCVS	NHPIFHEGSN	GTFIVTFDVS	Y----KATLG	888
CD11B	CESASSTEVS	GALKSTSCSI	NHPIFPENSE	----VTFNIT	FDVDSKASLG	893
CD11C	CCSA-PVGSQ	GTW-STSCRI	NHLIFRGGAG	----ITFLAT	FDVSPKAVGL	891

FIGURE 1C

αD	DRMLKRASAS	SENNKASSSK	ATFOLELPVK	YAVYTMISRQ	EESTKYFNFA	938
CD11B	NKLLLLKANVT	SENNMPRTNK	TEFOLELPVK	YAVYMMVTSH	GVSTKYLNFT	943
CD11C	DRLLLIANVS	SENNIPRTSK	TIFOLELPVK	YAVYIVVSSH	EQFTKYLNFS	941
αD	TS-DEKAKE	AEHRYRVNVL	SORDLAISIN	FWPVLNGV	AVMDVVM EAP	987
CD11B	AS-ENTS-RV	MOHQYQVSNL	GORSLPISLV	FLVPVRLNQT	VIMDRPQVTF	991
CD11C	ESEEKES-HV	AMHRYQVNNL	GORDLPVSIN	FWPVELNQE	AVIMDVEVSH	990
αD	SOSLP---CVS	ERKPPQHSDF	LTOISRSPHL	DCSIADCLQF	RCDVPSFSVQ	1035
CD11B	SENLSSTCHT	KERLPSSHDF	LAELRKAPVV	NCSIAVCORI	QCDIPFFGIQ	1041
CD11C	PQNPSLRCSS	EKIAPPASDF	LAHIQKNPVL	DCSIAGCLRF	RCDVPSFSVQ	1040
αD	EELDFTLKGN	LSFGWVRETL	OKKVLVWSVA	EITFDTSVYS	QLPGQEA FMR	1085
CD11B	EEFNATLKGN	LSFDWYIKTS	HNHLLIVSTA	EILFNDSVFT	LIPGQGAFVR	1091
CD11C	EELDFTLKGN	LSFGWVRQIL	OKKVSWSVA	EITFDTSVYS	QLPGQEA FMR	1090
αD	AQHEMVLEED	EVYNAIPIIM	GSSVGALLLL	ALITATLYKL	GFFKRHYKEM	1135
CD11B	SQTETKVEPF	EVNPIPLIV	GSSVGGLLLL	ALITAALYKL	GFFKRQYKDM	1141
CD11C	AQTITVLEKY	KVHNPIPLIV	GSSIGGLLLL	ALITAVLYKV	GFFKRQYKEM	1140
αD	LEDKPED----	-----TATFS	GDDFSCVAPN	VPLS		1161
CD11B	M---SEG----	-----GP--P	GAE-----PQ	----		1153
CD11C	M---EEANGO	IAPENGT--Q	TPS-----PP	SEK		1163

FIGURE 1D

## INTERNATIONAL SEARCH REPORT

In. ational application No.  
PCT/US94/14832

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.1, 240.2, 252.3, 320.1; 530/350, 387.1, 387.2, 388.1, 388.22; 536/22.1, 23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, CAS ONLINE, STN

search terms: human beta2 integrin, leukointegrin, leu.cam, leukocyte integrin#, alphad, integrin, alphas

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, Volume 72, issued 26 March 1993, Michishita et al., "A Novel Divalent Cation-Binding Site in the A Domain of the $\beta 2$ Integrin CR3 (CD11b/CD18) Is Essential for Ligand Binding", pages 857-867, see entire document.	1-38
A	CELL, Volume 69, issued 03 April 1992, Hynes, "Integrins: Versatility, Modulation, and Signaling in Cell Adhesion", pages 11-25, see entire document.	1-38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:		T	later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A	document defining the general state of the art which is not considered to be of particular relevance		
E	earlier document published on or after the international filing date	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means		
P	document published prior to the international filing date but later than the priority date claimed	Z	document member of the same patent family

Date of the actual completion of the international search:

11 APRIL 1995

Date of mailing of the international search report

17 APR 1995

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/14832

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF IMMUNOLOGY, Volume 150, Number 2, issued 13 January 1993, Fleming et al., "Structural Analysis of the <i>CD11b</i> Gene and Phylogenetic Analysis of the $\alpha$ -Integrin Gene Family Demonstrate Remarkable Conservation of Genomic Organization and Suggest Early Diversification during Evolution", pages 480-490, see entire document.	1-38

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/14832

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 19/00, 21/00; C12N 5/00, 15/00, 1/20; C12P 21/06; C12Q 1/68, 1/00; C07K 1/00, 2/00, 4/00, 14/00, 16/00;  
A61K 35/14

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 7.1, 69.1, 240.2, 252.3, 320.1; 530/350, 387.1, 387.2, 388.1, 388.22; 536/22.1, 23.1, 23.5

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